Preparation of low-dimensional materials for plasmonic bio-sensing

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2021

Das, C. M. (2021). Preparation of low-dimensional materials for plasmonic bio-sensing. Doctoral thesis, Nanyang Technological University, Singapore.
<https://hdl.handle.net/10356/154742>

<https://hdl.handle.net/10356/154742>

<https://doi.org/10.32657/10356/154742>

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PREPARATION OF LOW-DIMENSIONAL MATERIALS FOR PLASMONIC BIO-SENSING

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School of Electrical & Electronic Engineering

A thesis submitted to the Nanyang Technological University
in partial fulfillment 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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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.

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POENAR DANIEL PUIU

Authorship Attribution Statement

This thesis contains material from 7 paper(s) published in the following peer-reviewed journal(s) / from papers accepted at conferences in which I am listed as an author.

Chapter 4 is published as Das, C.M.; Ouyang, Q.; Dinh, X.Q.; Coquet, P.; Yong, K.-T., A theoretical insight into the use of anti-reflective coatings for the upliftment of sensitivity of surface plasmon resonance sensors. Optics Communications 2019, 458, 124748.

The contributions of the co-authors are as follows:

- A/Prof Yong K.T. gave the idea of performing the simulation study and assisted in the preparation of the final manuscript.
- I performed the entire simulation and Dr. Qingling Ouyang helped in verifying the results.
- I prepared the manuscript drafts. The manuscript was revised by Dr. Qingling Ouyang, Dr. X.Q. Dinh and Dr. P. Coquet.

Chapter 5 is published as Das, C.M.; Ouyang, Q.; Kang, L.; Guo, Y.; Dinh, X.Q.; Coquet, P.; Yong, K.-T., Anti- Augmenting Sensitivity of Surface Plasmon Resonance (SPR) Sensors with the Aid of reflective Coatings (ARCs). Photonics and Nanostructures – Fundamentals and Applications 2019, 38, 100760.

The contributions of the co-authors are as follows:

- A/Prof Yong K.T. gave the idea of performing the simulation study and assisted in the preparation of the final manuscript.
- Figure 1 was contributed by Dr. Lixing Kang.
- I performed the entire simulation.
- I prepared the manuscript drafts. The manuscript was revised by Dr. Qingling Ouyang, Dr. Lixing Kang, Dr. Yan Guo, Dr. X.Q. Dinh Prof. Philippe Coquet.

Chapter 6 is published as Das, C.M.; Guo, Y.; Kang, L.; Ho, H.P.; Yong, K.-T., Investigation of Plasmonic Detection of Human Respiratory Virus. Advanced Theory and Simulations 2020, 3, 7, 2000074.

The contributions of the co-authors are as follows:

- A/Prof Yong K.T. gave the idea of performing the simulation study and assisted in the preparation of the final manuscript.
- Figure 1 was contributed by Dr. Yan Guo.
- Figures 2-6 were contributed by Dr. Lixing Kang.
- I performed the entire simulation.
- I prepared the manuscript drafts. The manuscript was revised by Dr. H.P. Ho and A/Prof Yong K.T.

Chapter 7 is published as Das, C.M.; Guo, Y.; Guang, Y.; Kang, L.; Xu, G.; Ho, H.-P.; Yong, K.T., Gold Nanorod Assisted Enhanced Plasmonic Detection Scheme of COVID-19 SARS-CoV-2 Spike Protein. Advanced Theory and Simulations 2020, 3, 11, 2000185.

The contributions of the co-authors are as follows:

- A/Prof Yong K.T. gave the idea of performing the simulation study and assisted in the preparation of the final manuscript.
- C.M.D and Dr. Yan Guo contributed equally to the manuscript.
- Comsol simulations were performed by Dr. Yan Guo.
- All other sensitivity simulations were conceptualized by me and Dr. Yang Guang helped in running the simulations.
- Figure 3 was contributed by Dr. Lixing Kang.
- I prepared the manuscript drafts. The manuscript was revised by Dr. H.P. Ho, Dr. Gaixia Xu and A/Prof Yong K.T.

Chapter 8 is published as Kang, L.; Das, C.M.; Liu, D.; Chen, M.W.; Coquet, P.; Hong, G.; Yong, K.-T., A Comparative Performance Evaluation of 2D Nanomaterials for Applications in Plasmonic Biosensing. Physica Status Solidi A 2020, 217, 17, 2000255.

The contributions of the co-authors are as follows:

- A/Prof Yong K.T. gave the idea of performing the experiment and assisted in the preparation of the final manuscript.
- Dr. Lixing Kang and C.M.D contributed equally to the manuscript.
- Dr. Lixing Kang prepared the 2D material coated chips.
- Dr. Ming Wei Chen designed the experiment methodology and assisted in the operation of the commercial SPR machine.
- I prepared the manuscript drafts. The manuscript was revised and finalized by Prof. Philippe Coquet, Prof. Dan Liu, Prof. Guo Hong and A/Prof Yong K.T.

Chapter 9 is published as Das, C.M.; Kang, L.; Chen, M.W.; Coquet, P.; Yong, K.-T., Heterolayered Films of Monolayer WS₂ Nanosheets on Monolayer Graphene Embedded in Poly(methylmethacrylate) for Plasmonic Biosensing. ACS Appl. Nano. Mater. 2020, 3, 10, 10446-10453.

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- I prepared the manuscript drafts. The manuscript was revised and finalized by Prof. Philippe Coquet and A/Prof Yong K.T.

Chapter 10 is published as Das, C.M.; Kang, L.; Hu, D.; Guang, Y.; Guo, Y.; Chen, M.W.; Coquet, P.; Yong, K.-T., Graphene-Coated Gold Chips for Enhanced Goos-Hanchen Shift Plasmonic Sensing. Physica Status Solidi A Applications and Materials Science 2021, 2000690.

The contributions of the co-authors are as follows:

- A/Prof Yong K.T. gave the idea of performing the experiment and assisted in the preparation of the final manuscript.
- Dr. Lixing Kang and C.M.D contributed equally to the manuscript.
- Dianyí Hu prepared the graphene coated chips.
- Dr. Ming Wei Chen assisted in the biological experiment.
- I prepared the manuscript drafts and performed the optics experiment. The manuscript was revised and finalized by Dr. Yang Guang, Dr. Yan Guo, Prof. Philippe Coquet and A/Prof Yong K.T.

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Acknowledgement

I would like to express my sincere gratitude to my supervisor, Dr. Ken-Tye Yong and Dr. Poenar Daniel Puiu, for providing constant support and motivation to pursue research in the field of plasmonic bio-sensing. He has always encouraged me to explore different domains without having any doubts or fear of failure in mind.

Additionally, I would like to thank my parents, for always supporting me to pursue my dreams. I am thankful to almighty for giving me the opportunity to experience this PhD journey as this challenging pursuit presents many life lessons and at the end of each day it strives to make me a better person in all aspects

Abstract

Nano-bio-photonics is a unique blend of three primary disciplines namely nanotechnology, biological sciences and optics. Surface plasmons are free electron density waves that are a result of the photons interacting with the free electrons that reside on the surface of noble plasmonic metals like Gold (Au) and Silver (Ag). The field of plasmonic bio-sensing falls under this domain.

Plasmonic bio-sensors offer unique advantages over other sensors based on different methodologies like real-time, label-free detection, reusability of sensor chips and high accuracy results that are repeatable in nature. These sensors work on the basis of basic principle of Surface Plasmon Resonance (SPR) which is a physical phenomenon that occurs with the interaction between electromagnetic (EM) light wave and nano-sized plasmonic metals like Au and Ag.

Bio-molecular interactions that occur at the sensor surface have the ability to change the R.I. of the analyte medium. The change in this parameter produces a change in the optical parameter like the resonance angle, the phase shift of the reflected p -polarised light w.r.t. the s -polarised light or Goos-Hanchen (GH) shift between p and s polarised reflected light waves. Thus, a change in these optical parameters can be used to detect bio-molecular interactions occurring at the sensor surface.

The sensitivity of these SPR sensors play an important role in the precision and accuracy of detection of bio-molecular ligand-analyte or protein-protein interactions. Sensitivity in general can be defined as the differential change in optical parameter viz. the SPR angle, phase or GH shift w.r.t the change in the R.I. of the analyte medium. Conventional sensors employ the Kretschmann configuration which is 50 nm Au coated on a high R.I. prism.

A lot of research has been carried out on simulation that involves proposal of multi-layered SPR structures. However, most of these works propose structures that give good sensitivity results. In reality, simulation can be used only for preliminary assessment of multiple models where the output parameters can be compared for the sake of better understanding. Actual development of the proposed model will have many uncertain conditions like the quality of the nanomaterials fabricated, closeness and compactness of the SPR sensor packing, variation of refractive indices and thicknesses with temperature, pressure and mechanical stress that can lead to loss of sensitivity.

During the entire research duration of two and a half years, we focused on the use of simulation methods in predicting the sensitivity of SPR structures made of hybrid multi-layer low dimensional materials. We particularly focused on ARC materials like titania and silica and Transition Metal Dichalcogenides (TMDC) materials like WS_2 , MoS_2 , WSe_2 and $MoSe_2$. Although we use similar methods as given in previous related research works, we present a broader analysis where we evaluate the titania and silica sensor model at various wavelengths. Our Si-ARC-TMDC sensor model discussed in chapter 5 is an extension of a previously proposed Si-TMDC model. With an in-depth analysis of multiple sensor configurations, we highlight the importance of simulation as a basic step towards the realization and development of efficient sensors. Also, when the thickness of the dielectric layer(s) above and/or below the SPR metal film is below a threshold value of 3 nm, the non-locality effect comes into picture where the metal can no longer be represented by a bulk permittivity. In these cases, the evaluation method needs to be modified so that the non-local effect can be considered. Work that had been previously published did not consider such restrictions that are placed on the conventional method. Here, in this work, in addition to evaluating the sensitivity of multi-layered models, we also present a method by which the conventional procedure can be modified so that the sensitivity of plasmonic structures having extremely thin-layered dielectric coatings can be evaluated. Additionally, using commercial SPR sensor Biacore 3000, we evaluated the plasmonic capabilities of TMDC coated Au chips to detect protein-protein interactions. Many earlier research works have focused on the development of their own custom-made SPR sensors using TMDC materials like graphene and MoS_2 . Many such works promised functionalization-free sensing of bio-molecules. With such inspiration, with the help of commercial sensors we performed an initial assessment of multiple TMDC based arrangements to arrive at the best configuration that we used further for functionalization-free sensing of protein-protein interactions. With this our motive was to show how TMDC based functionalization-free SPR sensors can also be realized in the domain of commercial sensing platforms. Lastly, we performed some optical experiments (GH shift

sensitivity) to compare the sensitivities of pure Au and graphene coated Au chips. Very few researchers have focused on actual GH shift sensing experimental works and hence with this we intend to setup some initial basic work in this area that can be further utilized and developed in a better way in terms of experiment protocol and system design in order to realize effective GH shift based SPR sensors.

To conclude, our research work focuses on two aspects. The first is the theoretical approach where we use simulation in designing plasmonic sensor schemes and consecutively predict the sensitivities of those sensor layouts and the second part is where we focus on the experimental side where we perform both biological and optical experiments to evaluate the efficiency of plasmonic sensitivity enhancement capabilities of two-dimensional (2D) materials.

To conclude the entire research work and highlight the main achievements, following are the milestones that were achieved during the duration of the research period:

- A Titania and Silica-based SPR structure was proposed and the performance of the sensor was done using the Transfer Matrix Method (TMM). The best sensitivity of 214 deg/RIU was obtained at 532 nm for 40 nm Au, 3 nm SiO₂ and 9 nm TiO₂.
- An Anti-Reflective coating (ARC) and Transition Metal Dichalcogenides (TMDC)-based SPR structure was proposed that gave the best sensitivity of 284 deg/RIU at 633 nm for 50 nm Au, 95 nm TiO₂, 20 nm SiO₂ and 1.4 nm of WSe₂.
- A plasmonic structure using Au and Au + Graphene was realized that could be used for the detection of human respiratory viruses. The performance was analyzed at 690 nm, 780 nm and 930 nm. With the Au only layout, a minimum detection limit of 0.0669 HAU can be obtained at 690 nm and a maximum sensitivity of 500 deg/RIU and an average sensitivity of 122.4±38.68 deg/RIU can be obtained at 780 nm. With additional 15 layers of 0.34 nm Graphene, a minimum detection limit of 0.0188 HAU, maximum sensitivity of 1428.6 deg/RIU and an average sensitivity of 172.04±86.04 deg/RIU can be obtained at 690 nm.
- A Au nanorod-based sandwich type plasmonic structure scheme was proposed for the detection of SARS-CoV-2 virus. The electric field distribution was analyzed for various configurations of the Au NR. As compared to the Au only structure, the Au NR-based structure gave a maximum enhancement of 10.2 times when the aspect ratio of Au NR was four and it was located 2 nm from the Au nanosheet.
- A plasmonic performance comparison for various configurations of two-dimensional (2D) materials – Graphene, MoS₂ and WS₂ was studied using commercial SPR Biacore T200 sensor. The configuration of Au + Graphene + WS₂ gave maximum sensitivity enhancement of 4.66% as compared to only Au.
- Protein-protein interaction study between Bovine Serum Albumin (BSA) and Anti-BSA was studied using Biacore 3000 for Graphene-WS₂ modified Au chips. Functionalization-free bio-sensing of proteins could be achieved with a minimum detection limit of 0.44 µg/mL.
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Chapter 1

Thesis overview

1.1 Background

The bio-medical field is making continuous advancements and this has been possible with extensive work in the area of new sensing technologies. Plasmonic sensing is one such area that has been gaining huge popularity in the research fraternity. The broad area is classified into small domains of surface plasmon resonance (SPR) and localized surface plasmon resonance (LSPR) sensing as we shall see in the next chapters. However, the basic governing principle in these sensors remains the same. The basic testing methodology is based on the generation of surface plasmon polaritons (SPPs) that are electron density waves propagating at the surface of metal-dielectric interface. The detailed mechanism will be elaborated in chapter 3. However, in brief, the sensing arrangement requires a thin metal-dielectric interface which upon excitation by an electromagnetic (EM) light source generates SPPs that are the oscillations of the free electrons present on the metal surface. SPR-based detection can have distinct advantages of real-time and label-free detection. In addition to these, other features like reusability of chips, reproducibility of results and other benefits that shall be discussed later, the plasmonic testing scheme is superior in many aspects as compared to other non-optical testing methods.

A lot of advancements have been made in the area of point-of-care (POC) sensing technology and this has been possible with cutting-edge research in the area of SPR and LSPR sensing. Yuan et al. devised a fiber-optic SPR sensor that could detect heparin at low detection limits of 0.0257 ng/mL.¹ Nag et al. developed a drug susceptibility testing scheme using an LSPR-based optical fiber sensor.² Oh et al. designed an LSPR chip using Au nanoparticles (NP) that could detect *Salmonella typhimurium* in pork meats.³ He et al. could detect femtomolar limits of cancer biomarker in human serum samples with the help of graphene coated SPR chips.⁴ Harpaz et al. developed a plasmonic biosensor based on POC technology that could detect stroke biomarkers NT-proBNP and S100 β .⁵ POC testing kits especially for the detection of disease bio-markers is the need of the present generation. With many areas of the world that are still lagging behind in terms of socio-economic development, many rural areas in under-developed nations do not get access to proper medical care and diagnostics. POC testing devices can be extremely useful in such scenarios as compact yet highly sensitive testing kits can serve the purpose.⁶⁻⁷

Thus, in the years to come, further investment and advances in the area of plasmonic sensing research can help to bridge the gap between the developing community of the world and their access to modern-day medical facilities. The future of the bio-medicine community needs highly sensitive and selective plasmonic sensors that are compact, robust and resilient. Hence, this research work delves into the domain of SPR sensing and revolves around the concept of using low dimensional materials for the purpose of enhancing the sensitivity of SPR sensors.

1.2 Motivation & Objective

As we shall see further, the thesis has been divided into two parts: the first part is on simulation and the second part is on experimental work. The thesis is about the use of low dimensional materials for the purpose of plasmonic sensing. Hence, in chapters 4 through 7, we make use of simulation as a tool to have an in-depth look on how the structural arrangement of various nano-sized layers can be optimized to get the desired layout that gives the best sensitivity. The primary objective here is to show how simulation can be used effectively to design surface plasmon resonance (SPR) sensing models and also compare the performance of various models in order to arrive at the optimal configuration. Most of the earlier research works that use simulation do not discuss the concept of non-locality that can arise when the adjacent layer to the plasmonic metal is extremely thin, namely less than 3 nm when the sensitivity can no longer be evaluated using the standard method. Thus, the aspect of non-locality has also been discussed here and subtle procedural and sensitivity evaluation changes have been detailed that can help tackle the special case of extremely low dimensional materials surrounding the plasmonic metal. In the second part actual optical and bio-sensing experiments have been performed using two-dimensional (2D)

material coated Au chips. The bio-molecular sensing experiments have been performed using a commercial SPR machine. Many earlier research works have been performed to test the efficacy of 2D materials in functionalization-free sensing. However, most of these have been done using customized set-ups that have been designed and modelled by the respective researchers and are not in commercial use. Thus, by taking motivation from such works, the bio-molecular experiments performed here on commercial sensors show that 2D materials can be beneficial in the development of a different bio-molecular SPR testing regime and protocol that does not need complex functionalization schemes to be executed. The objective of the optical experiment in Chapter 10 was to show that the concept of Goos-Hanchen (GH) shift of SPR sensing can be extended experimentally and thus with a naive customized arrangement, GH shift sensing experiments were carried out on graphene coated chips.

1.3 Contribution & Originality

The thesis chapters 4 through 10 are published as journal papers. The simulation structure ideas in chapters 4 to 7 are completely original and authentic. Additionally, the experimental work in chapters 8 through 10 are also original with the basis derived from earlier research works. The actual contribution by other co-workers and co-authors have been detailed out in the authorship attribution statement.

1.4 Structure of the thesis

In the following chapters, we first review some research work performed in the area of plasmonic bio-sensing in chapter 3. The basic aim to review these is to get an idea on how these works serve as the motivation and basis for carrying out further research in the domain of SPR sensing. Coming to the main content, the thesis is divided into two parts: part 1 focuses on using simulation as a tool to predict sensitivity of multi-layered SPR structures and part 2 focuses on bio-sensing experiments performed using commercial Biacore SPR instrument and optical experiment performed in a customized setup. The main aim of part 1 is to show how simulation can serve as an important medium for setting up and then consecutively analyzing the efficiency of the multi-layered structure comprising of low dimensional materials. Part 2 has been used to show that in reality low dimensional materials can actually give a boost to the sensitivity of SPR structures. The motivation of part 1 is to show how simulation is an indispensable tool for the initial preliminary assessment of sensor structures and configurations. Although many research works have presented many sensor models, we give an in-depth analysis on how sensitivity is actually a complex phenomenon involving many factors of refractive index, quality and dimension of nanomaterials and wavelength. We also extend our discussion by introducing the concept of non-locality that arrives when extremely low dimensions of dielectric materials are used as the layer adjacent to the plasmonic metal. We present a method by which a small change in the simulation model can help us in taking care of the non-locality. We believe that such additions are unique here as most earlier works don't discuss about these.

In part 2, our aim is to take inspiration from some earlier works on customized SPR models used for protein-protein interaction and extend it to commercial sensing devices where we show how the commercial SPR sensing models can be changed to 2D material-based Au chips for the easiness in the preparation of sensing modules and testing procedures. We also perform Goos-Hanchen shift experiments to show that the theoretical phenomenon which has been discussed by many researchers can also be demonstrated practically.

It is a well-known fact that the RI of water is dependent on the temperature. In real-case scenarios, the detection limit does not improve much beyond a certain limit because of this fluctuation of RI with temperature and the inability to control temperatures precisely results in a drifting baseline that can cause detection of low concentrations getting nullified in the background noise. However, the change in RI is very nominal. In our simulations, we have considered the RI as 1.33 as the range of variation of RI is quite short (1.33 to 1.337) in the visible spectrum of the EM wave. Also, at a particular wavelength, it requires almost 6–7 °C change of temperature to cause a change of 0.0001 RIU change in the RI of water.⁸ Thus, for nominal <1 °C changes in temperature, the RI can mostly be considered constant for ease of calculation.

For the experiments in chapters 8 and 9, these have been carried using a professional equipment Biacore 3000 and thus the machine has its own temperature regulation mechanism. For those experiments, the temperatures were maintained and controlled throughout the experiment as the machine has its own signaling mechanism that notifies the user of any fluctuations. For the experiment in chapter 10, it was

carried using a customized setup and did not involve any commercial equipment but mostly relied on human interaction. However, these experiments were carried out in a controlled environment where the temperature regulation was good and thus any temperature change beyond 0.5 to 1 °C would be highly unexpected.

Chapter 2

Introduction

2.1 Surface Plasmon Polaritons (SPP)

Figure 2.1 Refraction of light at the interface of two materials.⁹

Surface Plasmon Resonance (SPR) was first observed by Wood at the start of the 19th century. At that time, it was simply regarded as just another physical phenomenon. But today, many chemical and biological sensing techniques have incorporated this simple yet efficient principle as their basic mode of operation. Plasmons are essentially a result of light photons interacting with free electrons that reside at the surface of nano-sized noble metals like Gold (Au) or Silver (Ag). In the field of plasmonic sensing, these metals are also known as plasmonic metals as upon the fulfillment of certain mandatory conditions, they show the SPR effect.¹⁰⁻¹³

The topic of surface plasmon resonance (SPR) starts with its most basic entity – the surface plasmon polaritons (SPP), its properties and how these are excited.⁹ For the generation of SPPs, the essential condition is the presence of free electrons on the at the interface of two materials. In actuality, one of these is a metal that has large amounts of free conduction electrons. Thus, SPPs are electron gas clouds that oscillate with respect to their fixed ionic cores. Alternatively, these are EM waves that are strongly bound to the interface. Before we explain the mechanism behind SPR, let us look at the evanescent wave generated at the single metal-dielectric interface.

Figure 2.1 shows the refraction taking place between two media of refractive indices n_1 and n_2 . The EM wave propagating in a medium of R.I. n can be given as⁹

$$E = E_o \exp(\omega t - jk \cdot r) = E_o(\omega t - jk_x x - jk_y y - jk_z z) \quad (2.1.1);$$

E_o is the amplitude of the electric field, ω is the angular frequency, k is the wavevector and r is the position vector. The direction of k is parallel to the direction of wave propagation and is given by⁹

$$k = \sqrt{k_x^2 + k_y^2 + k_z^2} = n \frac{2\pi}{\lambda} = n \frac{\omega}{c} \quad (2.1.2);$$

c is the velocity of light in vacuum and λ is the wavelength of the field. While defining refraction taking place between the two media above in Figure 2.1, the direction of light beam is chosen such that $k_z = 0$ so that the analysis becomes easier with two-dimensional approach. Here, α and β are the angle of incidence and refraction respectively.⁹ From Snell's Law

$$n_1 \sin \alpha = n_2 \sin \beta \quad (2.1.3);$$

$$k_{x_1} = k_{x_2} \equiv k_x \quad (2.1.4);$$

Using 2.1.2 and 2.1.4, the component of wavevector perpendicular to the interface can be given as⁹

$$k_{y_2}^2 = n_1^2 \left(\frac{2\pi}{\lambda} \right)^2 \left(\frac{n_1^2}{n_2^2} - (\sin \alpha)^2 \right) \quad (2.1.5);$$

When $n_1 > n_2$, which is generally the scenario in metal-dielectric interfaces, k_{y_2} is purely imaginary. Hence, for medium 2, the travelling wave is parallel to the interface and is given by⁹

$$E_2 = E_0 e^{-\kappa_{y_2} y} \exp(j\omega t - jk_x x) \quad (2.1.6);$$

The amplitude of the field is exponentially decaying along the y -direction with a characteristic distance of $1/\kappa_{y_2}$ or $1/jk_{y_2}$ and is thus known as the evanescent field because of its decaying nature. Now we shall discuss about the SPP dispersion equations. For a two-media interface, the complex reflection coefficient is given by Fresnel's equations as shown below⁹

$$r_p = \frac{E_i}{E_r} = |r_p| e^{j\varphi} = \left| \frac{\tan(\alpha - \beta)}{\tan(\alpha + \beta)} \right| e^{j\varphi} \quad (2.1.7);$$

E_i and E_r are the incident and reflected electric fields and the angles α and β are as shown in Figure 2.1. If $\alpha + \beta = \frac{\pi}{2}$, the denominator of r_p becomes large and it approaches zero. If $\alpha - \beta = \frac{\pi}{2}$, r_p is infinite or for a very small value of E_i there is a finite E_r and this corresponds to resonance. Thus, we can say that $\cos \alpha = -\sin \beta$ and $\tan \alpha = \frac{k_{1x}}{k_{1y}} = -\frac{n_2}{n_1}$. For the wave vector given by $k = (k_x, k_y)$, we can write the following⁹

$$k_x^2 = k_1^2 - k_{y_1}^2 = k_1^2 - k_x^2 \frac{\varepsilon_1}{\varepsilon_2} \quad (2.1.8);$$

$$k_x = \frac{\omega}{c} \sqrt{\frac{\varepsilon_1 \varepsilon_2}{\varepsilon_1 + \varepsilon_2}} \text{ and } k_{yi} = \frac{\omega}{c} \sqrt{\frac{\varepsilon_i^2}{\varepsilon_1 + \varepsilon_2}} \quad (2.1.9);$$

ε_1 and ε_2 are the dielectric constants of the two media and $i=1$ or 2 . Equation 2.1.9 gives the SPP dispersion equation for an interface between two half-infinite media. For the case, when medium 2 is a metal the following equations apply⁹

$$\varepsilon_2(\omega) = 1 - \frac{\omega_p^2}{\omega^2} \quad (2.1.10);$$

$$\omega_p = \sqrt{\frac{4\pi n_e e^2}{m_e}} \quad (2.1.11).$$

ω_p is the plasma frequency, n_e is the free electron density, e and m_e are the electron charge and mass of

Figure 2.2 Dispersion relation for surface plasmons. Reproduced with permission.⁹

electron respectively. For $\omega < \omega_p$, no EM field can propagate in a metal and for $\epsilon_2 > -\epsilon_1$, k_{yi} is imaginary and k_x is real. Hence, the EM field propagates along the interface and the evanescent part extends into both sides of the interfaces. The dispersion relation between SPPs can be understood by the following figure.⁹

In figure 2.2 curve I shows the dispersion relation in equation 2.1.9 for the interfaces ϵ_3/ϵ_m and curve II for the interface ϵ_1/ϵ_m . Curve (a) shows the dispersion relation for “normal” light. If we consider just two interfaces, then the only point of intersection for the two curves I and (a) is origin. Thus, for a two-medium layout, normal light cannot be used for the excitation of SPs. When a thin metal layer is inserted between the two mediums, two dispersion relations can be observed curves I and II. The dispersion relation for normal light in medium 1 now intersects curve I or the SP dispersion for metal/medium 3 interface. Hence, by proper adjustment of incident angle α , the incoming wave-vector can be tuned to match the SP wave-vector for the excitation of SPP. Any k_x between the two lines labelled a and b can be set to excite the plasmons. The attenuated total refraction (ATR) configuration that was first demonstrated by Kretschmann and Rather is the widely used standard method for SP excitation and will be discussed in the next section.

The main properties of the SPP are: field enhancement, phase jump of the reflected light and SP coherence length. The evanescent at the low RI side of the metal is much higher than the other side of the metal layer. Also, at an angle close to the resonance angle, the field is enhanced to as much as upto 30 times in comparison to the intensity at other incident angles. Moreover, at near about resonance angle, the phase of the reflected electric field undergoes a large change. A small change in R.I. of the sensing layer can result in large phase jumps giving rise to high phase sensitivity. However, the experimental setup to measure such changes is more complicated than the standard angular sensitivity measurement. The metal dielectric constant is complex and this gives rise to complex propagation constant given by $k_x = \kappa_x' + j\kappa_x''$ where κ_x' and κ_x'' are the real and imaginary parts of the wave vector k_x . For a SPP that travels on the interface, the field intensity decays with a characteristic length of $1/2\kappa_x''$. In case of standard plasmonic metals like gold and silver, the imaginary part of the dielectric constant increases with decreasing wavelength and the SP propagation length decreases accordingly. Many experiments conducted previously suggested that a plasmon roughly needs four times the propagation length $L_x = 1/2\kappa_x''$ for a full decay. Hence, SPPs with mutual distances greater than L_x are considered to be independent. This forms the basis of many SPR applications like SPR imaging, multisensing and

Figure 2.3 Schematic of the Kretschmann configuration for SPR sensing

microscopy. Specific independent areas can be defined on the substrate provided that these areas have surface areas larger than L_x^2 .

2.2 Theory of Surface Plasmon Resonance

Surface Plasmon Waves (SPW) are electromagnetic waves that travel along the plasmonic metal-dielectric interface. The SPR effect is observed when *p*-polarized light wave (LW) or light propagating in the transverse magnetic (TM) mode, falls on the metal-dielectric interface. In order to excite the plasmons, it is mandatory that the wave-vector of the incident light is parallel to the interface and matched with that of the SPW. When light is allowed to fall directly on the plasmonic metal surface, the SPR effect cannot be observed as the propagation constant of the SPW is higher than that provided by the incident LW vector. Hence, in order to observe this physical principle, special arrangements are required so that the two wave vectors can be made equal.

One of the most important and necessary conditions for the generation of plasmons is the matching of wave vectors. Only when the component of the incident light wave vector (k_x) parallel to the metallic surface matches with that of the SPW (k_{sp}), the resonance condition is achieved and plasmons are generated. The matching of the wave vectors can only be achieved with the help of special arrangements like the Attenuated Total Reflection (ATR) configuration. The attenuated total reflection (ATR) method makes use of an optically dense prism to excite the plasmons. The Kretschmann arrangement is a very well-known setup based on the ATR procedure which utilizes a high refractive index (R.I.) prism to enhance the wave vector of the incident LW. A typical Kretschmann configuration is shown in Figure 2.3. This comprises of three layers namely a glass prism (SF-10, SF-11 or BK7), 50 nm thick gold layer coated on top of the prism and the dielectric layer (air or de-ionized (D.I.) water).

Figure 2.4 represents the basic SPR curve for the 50 nm Au Kretschmann configuration based on BK7 glass prism and D.I. water as the dielectric medium at an incident light wavelength of 633 nm. The curve depicts the relation between reflectivity and the incident angle. The reflectivity is the ratio of the intensities of the incident and reflected light waves as shown in Figure 1.1. Since the light wave travels from an optically dense prism to another medium with a low R.I., when the incident angle is greater than the critical angle, the light is total internally reflected. As observed in the SPR curve, after the critical angle is reached the reflectivity starts to drop till a point where it is minimum and upon further increase of incident angle, the reflectivity starts to increase. The angle at which reflectivity is minimum is known as the SPR angle as at this resonant condition, the matching of the two propagation constants takes place. The mathematical relation explaining this condition is shown below in equations 1.1-1.3. K_x and k_{sp} are the propagation constants of the incident LW and the SPW respectively. n_1 , n_2 and n_3 are the refractive indices of the three media, the glass prism, the plasmonic metal Au and the dielectric layer (air or de-

Figure 2.4 Basic SPR reflectivity curve for Kretschmann arrangement at 633 nm wavelength

ionized (D.I.) water).¹⁴

$$k_x = \frac{2\pi}{\lambda} \times n_1 \times \sin \theta_{res} \quad (2.2.1);$$

$$k_{sp} = \frac{2\pi}{\lambda} \times \sqrt{\frac{n_2^2 n_3^2}{n_2^2 + n_3^2}} \quad (2.2.2);$$

Equating the two equations, the resonance angle can be found out.

$$\theta_{res} = \arcsin Re \left(\frac{1}{n_1} \sqrt{\frac{n_2^2 n_3^2}{n_2^2 + n_3^2}} \right) \quad (2.2.3).$$

2.3 Sensitivity of SPR Sensors

SPR bio-sensors are quite popular nowadays and they have been used for many pathological applications like the detection of *Salmonella* spp, a bacteria and quantification of Troponin T, a protein found in cardiac and skeletal myocytes and many more.¹⁵⁻¹⁶

Immunosensing is based on specific antigen-antibody or ligand-analyte binding taking place on the sensor surface. This specific interaction leads to a physicochemical change that can be converted to a quantifiable output signal. SPR based immunosensors have garnered huge popularity in bio-medicine as they offer real-time and label-free detection methodology. Apart from these, they can easily operate with small volume samples, avoid wastage of consumables due to re-usability of sensor chips and the results are accurate and repeatable. SPR immunosensors rely on small R.I. changes occurring because of specific binding or interaction between the antigen and antibody pair. This physical change can be converted to a readable output signal. As seen in equation 2.2.3, the SPR angle is highly dependent on the R.I. of the

Figure 2.5 Concept of sensitivity enhancement using stacking of nano-sized dielectric materials as intermediate layers

analyte medium n_3 . Small changes in R.I. can cause a shift in the resonance angle. Thus, when bio-molecular interactions occur in the analyte medium, they cause the R.I. of the medium to change. Hence, with the help of this shift in physical property viz. the resonance angle, it is possible to detect, analyze and quantify the interaction level between the antigen and antibody pairs.^{10, 17-21}

Sensitivity can be defined as a change in the quantifiable parameter (angle, phase, wavelength or Goos-Hanchen (GH) shift) occurring as a result of a change in the R.I. of the analyte medium. Most commercial sensors utilize angular interrogation technique where the ligand-analyte interactions cause a change in the resonance angle. Angular sensitivity, $S_{angular}$, can be defined by equation 2.3.1 and this is one of the most convenient methods to configure the output response as compared to other techniques that require complex optical setups.¹⁴

$$S_{angular} = \frac{d\theta_{res}}{dn_3} \quad (2.3.1).$$

2.4 Concept of sensitivity enhancement

Sensitivity plays an important role in the precision and ease at which bio-molecular interactions can be detected. As the complexity of these interactions increase, it is highly desirable to have an enhanced sensing system that has lower detection limit capabilities. Researchers have proposed many new models and sensor configurations that can have enhanced sensing capabilities compared to the standard Kretschmann arrangement. Most of them have done this by placing nano-sized sheets of suitable dielectric materials between the plasmonic metal and the analyte. The reason behind greater sensitivity is higher light-matter coupling between the incident photons and the plasmonic metal that promotes the generation of more surface plasmons. The idea behind sensitivity enhancement has been depicted in Figure 2.5.

2.5 Evanescent Field

Plasmonic oscillations result in SPWs that are electromagnetic (EM) waves which travel at the metal-dielectric interface. The resultant electric field has two components, one parallel to the interface and the other perpendicular to the interface. The normal component is known as the evanescent field since it is exponentially decreasing in nature. The value of this component is highest at the metal surface and it

decreases as one traverses through various layers towards the analyte. The evanescent component vanishes after travelling about one-third to half wavelength distance from the surface. The penetration depth, L_p , of this field is defined as the distance at which the electric field is 1/e of its maximum value at the plasmonic metal surface. Sensitivity enhancement has a direct correlation with the enhancement in this field. Bio-molecular interactions that occur within the penetration depth have higher chances of being detected. Thus, when the value of this evanescent field increases, there is a greater possibility of the depth of penetration getting enhanced which improves the chances of detection.²²⁻²⁴

2.6 Localized Surface Plasmon Resonance (LSPR) Sensing

It was already shown in previous section 2.1 that plasmons are collective oscillations of free electrons in a noble metal. These can be thought of as oscillations of the electron gas of a metal. The presence of an external electric field displaces the electron gas with respect to fixed ionic cores. Surface plasmons can be optically excited by coupling the incident light into standing or propagating surface plasmon modes with the help of a grating or a defect on metal surface. When a surface plasmon is limited and constrained within the size of a particle comparable to the wavelength of light, a nanoparticle, the free electrons of the NP oscillate collectively when excited by external field, and this oscillation is termed as localized surface plasmon (LSP). Two major effects take place here, first the electric fields near the surface of the NP are magnified several times. Similar to the SPR in metal nanosheets, the LSP field is maximum at the surface of the NP and it gradually reduces and disappears with increasing distance. Secondly, the NPs optical extinction is greatest at the LSPR frequency and for noble metals this occurs at visible wavelengths. The extinction peak is dependent on the R.I. of the surrounding region and thus forms a basis for sensing applications.²⁵⁻²⁹

The LSPR peak wavelength is dependent on the dielectric function of the medium and thus most R.I. sensing devices that use the principle of LSPR monitor the change in the peak wavelength to understand and gauge the changes happening in the surroundings of the NP. The LSPR peak wavelength at visible and NIR frequencies is denoted by the following equation. This equation is a simplified version derived from the Drude model of electronic structure of metals.²⁶

$$\lambda_{max} = \lambda_p \sqrt{2n_m^2 + 1} \quad (2.6.1).$$

where λ_p = Wavelength corresponding to plasma frequency of metal and n_m = Refractive index of the surrounding medium.

The many applications of LSPR sensing include refractive-index sensing, molecular sensing and even LSPR spectroscopy. In comparison to the evanescent field decay observed in metal nanofilms, the rate of decay in case of LSPR sensors is extremely fast. The sensing region is limited to only about 10-20 nm region surrounding the NP in comparison to 100-200 nm in the case of thin film SPR sensors. Thus, LSPR sensors are extremely useful in detecting local changes in R.I. that occur near the surface of the NP. In contrast, SPR sensors are useful for bulk R.I. sensing. We shall separately look into some SPR and LSPR-based bio-detection schemes in chapter 3.

Film-based SPR sensors have come to the forefront in the bio-medical industry where they are able to detect a plethora of bio-molecules like cancer and inflammatory disease bio-markers. Their optically compliant behavior may cater for commercial sensing systems; however, they do not have the ability to be transformed into a portable system because of their size limitation. In contrast, LSPR systems are miniaturized sensors that work on the same principle as film-based SPR sensor, but because of their basic structural difference they can be easily designed in the form of a portable lab-on-a-chip format. Another advantage of the LSPR sensing method is that the LSPR peak frequency can be fine-tuned by changing the shape and size of the NP like by varying the aspect ratio of Au NRs. This can be useful in the case of real-time biological sensing of samples like serum, when certain frequencies, where naturally occurring chromophores can absorb and extinct the response signal, can be avoided.

LSPR sensors have proved to be a boon to the portable sensing industry. However, as compared to film-based sensors, they have low penetration depths. The sensing range of film-based SPR sensors varies from 100 to 200 nm. In contrast to this, LSPR sensors have one-tenth of the sensing range as the evanescent field vanishes within 10-20 nm from the NP sensing surface because of the local nature of the plasmonic effect. This can pose serious challenges while designing the sensor. Extreme care needs to be taken during functionalization and even immobilization of ligands or specific antibodies as all of these

add up together and can extend the analyte layer from the sensing surface. Hence, proper design of the LSPR sensor where optimal distance is maintained between the analyte and the sensing region is of prime importance.

Thus, to summarize both SPR and LSPR sensing, in spite of their pros and cons, have their own unique values in the field of biological detection. LSPR sensing technology can be utilized for point-of-care (POC) testing when the molecules of interest can be easily detected within the localized volume of sensing. On the other hand, more complex detection schemes that require functionalization need a robust testing approach that utilizes SPR technology since it has a bulk sensing volume that enhances the sensing region.

2.7 Conclusion

SPR sensors have revolutionized the field of biological sensing. In this chapter, we presented a brief introduction on the physical principle behind this phenomenon and also introduced some concepts on sensitivity enhancement. In the following chapters, we shall go in to the details of analyzing the sensitivity and output performance of multi-layered SPR sensors with the aid of computation tools. We shall also discuss some results obtained by performing actual bio-molecular experiments using both commercial and custom-made SPR sensors.

Chapter 3

Literature review

In this chapter we shall take a look at the various works that many researchers have focused on in the area of plasmonic bio-sensing both in the domain of simulation studies and experimental works. The basis of our simulation work has been inspired from these research works that have been previously performed. Also, our experimental studies have been designed based on the research works that we shall briefly discuss here.

3.1 SPR and LSPR bio-detection devices

We discussed about the concepts of SPR and LSPR-based sensing in the previous chapter. A lot of work has been done in this area and is still being done. Many researchers have developed their own customized setup to detect different kinds of biomolecules. Liu et al. developed their own SPR scheme to detect immunoglobulin G (IgG). They utilized a unique setup comprising of red-green dual-color fiber optic SPR sensor integrated with a smartphone that could monitor the red and green channels. It had a linear response in a RI range of 1.326 to 1.351 ($R^2 = 0.991$) and had a resolution of 2.3×10^{-4} RIU. Linear SPR response could be achieved for concentrations between 0.02 to 0.3 mg/mL. Because of a simple detections scheme along with other factors of reasonable detectivity and portability, such SPR schemes can be highly useful for POC detection.³⁰ Tawil et al. utilised a charge-coupled device (CCD) camera-based SPR setup for the detection of *Escherichia Coli* (*E. Coli*) and methicillin-resistant *Staphylococcus aureus* (MRSA). The system could effectively detect small concentrations of 103 colony forming units/millimeter in a short span of about 20 minutes.³¹ In another unique application, Meneghello et al. used a grating coupled SPR (GC-SPR) scheme for enhanced spectroscopy method of detecting mutations that cause cystic fibrosis.³² Figure 3.1 (a), (b) and (c) shows a schematic of the SPR setups mentioned above.

Coming to some beautiful and extraordinary LSPR schemes developed by researchers, Wei et al. used a miniaturized fiber optic (FO) nanobiosensor, based on Au nanodisk arrays showing LSPR, for the detection of protein biomarkers like prostate specific antigen (PSA). The device could achieve low levels of detection at 100 fg/mL.³³ Neuzil et al. designed a unique POC device that was supported by a battery, an LSPR chip, four pulse-powered light emitting diodes (LEDs) and a photodiode. They demonstrated the utility of their device by measuring the LSPR absorption peaks for different concentrations of ethanol.³⁴ Wang et al. devised a glucose sensor that measures the shift of the LSPR peak due to varying concentrations of hydrogen peroxide (H_2O_2) generated as a by-product of oxidation of glucose with the help glucose oxidase (GO_x) that acts as a catalyst.³⁵ Figure 3.2 (a) shows the schematic of the LSPR setup used for the detection of PSA, figure 3.2 (b) shows the schematic of the battery-powered POC device and figure 3.2 (c) shows the LSPR peaks of Au NRs for different concentrations of H_2O_2 generated as a result of glucose oxidation.

3.2 2D materials for plasmonic sensing

2D nanomaterials can be defined as atomically thin materials such as graphene, MoS_2 , WS_2 , $MoSe_2$, WSe_2 , $PtSe_2$, antimonene and many more. The low dimensionality of these materials confers them new properties or affects some of their existing physical properties normally exhibited in the bulk of the same macroscopic materials. These new or modified properties make them very useful for optical sensing applications. For instance, due to their electronic structure, they can easily adsorb molecules usually mediated by p- p interactions or by van der Waals interactions. Another important benefit conferred by 2D materials relates to functionalization. As was mentioned previously, most conventional SPR sensing schemes require complex functionalization processes in order to attach the ligand molecules to the plasmonic metal surface through certain linkers. However, with the recent advancements in the discovery of transition metal dichalcogenides (TMDCs), many researchers used materials such as MoS_2 , WS_2 and antimonene to design functionalization-free SPR sensors to detect biomolecules like Bovine Serum Albumin (BSA) and micro Ribonucleic Acid (mi-RNA).³⁶⁻³⁸

(a)

(b)

(c)

Figure 3.1 (a) Schematic of the smart phone-based dual color fiber-optic SPR sensor³⁰ (b) CCD-camera based SPR setup³¹ (c) Schematic of the experimental configuration geometry for GC-SPR-based detection.³²

Figure 3.2 (a) Schematic of the Au nanodisk based LSPR sensor³³ (b) Schematic of the portable LSPR sensor powered by a battery³⁴ (c) LSPR peaks of Au NRs for different concentrations of H_2O_2 generated a: 0.1 b: 1 c:10 d:100 nM.³⁵

Figure 3.3 (a) Fabrication process of a miRNA sensor integrated with antimonene nanomaterials.³⁷ (b) Schematic of the fiber optic SPR setup for *E. coli* detection.³⁶ (c) Schematic of the optical fiber SPR biosensor developed with and without MoS₂ nanolayer.³⁸

An additional advantage associated to the extraordinary properties of these 2D materials is that the optical energy of the incident photons can be transferred more efficiently to generate the SP wave because of the high carrier mobility in some 2D materials, such as Graphene, MoS₂ and WS₂ which can result in highly sensitive SPR sensors. Moreover, most of the current 2D materials are biocompatible and thus the risk of structural deterioration and denaturation of biomolecules is greatly reduced.³⁹⁻⁴¹ In addition to these advantages, most 2D materials especially graphene and some TMDCs have a broadband light absorption that enables the SPR sensors incorporating these materials to operate even at NIR and IR wavelengths.⁴²⁻⁴⁴

Figure 3.3 shows some schematics of the experimental setup and the fabrication process of some 2D material based SPR sensors that have been designed by researchers. Figure 3.3 (a) shows the fabrication process of a miRNA sensor based on antimonene and figure 3.3 (c) shows the method for fabricating MoS₂ chips that were used for functionalization-free sensing of bovine serum albumin (BSA). Figure 3.3 (b) shows the experimental setup of the fiber optic SPR sensor used for detection of *Escherichia Coli* (*E. Coli*).

3.3 Sensitivity Enhancement

As the complexity of biomolecules increase, it is essential to develop sensor structures that have high sensitivity and also have lower detection limits. Thus, researchers have used various nano-sized materials and have formed multi-layered SPR structures using them and have performed simulation study to analyze the sensitivity of the SPR structure. Wu et al. used multi-layered graphene and designed a SPR sensing scheme that was $(1+0.025L)\gamma$ times more sensitive than the conventional Kretschmann configuration, where L and γ are the length and adsorption factor of graphene respectively.⁴⁵ In brief, each layer of graphene was 0.34 nm and a total L layers were placed on top of 50 nm Au. They performed the simulation and verified the sensitivity by varying L. Recently, researchers have focused on a new class of materials called Transition Metal Dichalcogenides (TMDCs) and they have found it to be quite effective. For instance, Zeng et al. used graphene and MoS₂ to form a modified sensing scheme.⁴⁶ Briefly, the structure comprised of 0.65 nm of MoS₂ deposited on top of Au film and then consecutively followed by 0.34 nm of graphene. Moving further, Maurya et al. designed a composite structure where they placed TiO₂ and SiO₂ in between the glass prism and the Au film, and MoS₂ and graphene on top of the Au film.⁴⁷ They observed that the sensitivity could be enhanced by 12.82 % and 3.28% as compared to the conventional SPR and graphene-MoS₂-based sensing scheme respectively. Verma. et al. used Cr and Ag and designed a complex sensing scheme comprising of airgap-Cr-Ag-graphene as the intermediate layers in between the prism and the sensing medium.⁴⁸ The sensor proposed by them gave 2.35 times enhancement in sensitivity as compared to the graphene-based sensor without any air gap. Additionally, Ouyang et al. used four different types of TMDCs, MoS₂, MoSe₂, WS₂ and WSe₂ and performed the simulation at varied wavelengths.⁴⁹ In brief, they placed silicon and the TMDC material MX₂ on top of the Au film to find out the most effective combination that could give the highest sensitivity at different wavelengths. Moreover, Kushwaha et al. designed a multi-layered scheme using ZnO, MoS₂ and graphene. They placed the ZnO layer in between the prism and the Au film and the MoS₂ and graphene layers were placed over Au.⁵⁰

Moving ahead, other researchers designed additional SPR sensing structures. Kushwaha et al. used ZnO and graphene where they placed ZnO in between prism and Au film and Graphene on top of Au film.⁵¹ Lin et al. used two layers of Au and MoS₂ and graphene.⁵² The intermediate layers were Au-MoS₂-Au-graphene. Wu et al. used Al as the plasmonic metal and designed a structure with air gap-MoS₂-Al-MoS₂-graphene as the intermediate layers.⁵³ Nisha et al. used Au, MoS₂, Ni and graphene and designed a SPR sensor with MoS₂, Ni and graphene on top of Au.⁵⁴ Sun et al. placed BaTiO₃ and graphene on top of Ag.⁵⁵ Dai et al. used SnSe and placed it over Ag.⁵⁶ Figure 3.4 shows the schematic of some of the proposed schemes discussed here. In chapters 4 and 5, we shall compare the sensitivity and performance of our model with the various schemes discussed here.

Thus, to conclude we can see that the researchers placed different nanomaterials of various thicknesses over the plasmonic metal. Many researchers even used other plasmonic metals like Ag and Al and different kinds of glass prism materials like SF10, SF11 and BK7 were utilized. We utilized the same concept and, in our simulation work that we discuss in chapters 4 and 5, we incorporated materials like TiO₂, SiO₂, Nb₂O₅, MgF₂ and TMDC materials like MoS₂, MoSe₂, WS₂ and WSe₂ and placed the materials taking many different combinations.

(a)

(b)

(c)

(d)

Figure 3.4 Schematic of the SPR schemes based on (a) Graphene and MoS₂⁴⁶ (b) Si and WS₂⁵⁷ (c) ZnO and Graphene⁵¹ and (d) ZnO, Graphene and MoS₂⁵⁰

3.4 Anti-reflective Coatings

When it comes to optical or opto-electronic efficiency of devices like liquid crystal display (LCD) screens, solar photovoltaic cells or light emitting diodes (LEDs) that are made up of multiple layers of contrasting materials, conventional design can cause significant reflection losses that arise due to a stark contrast between the refractive indices of the constituent layers.⁵⁸⁻⁶⁰ Transparent optical materials can face reflection losses from both sides, for the incoming and outgoing light. Anti-reflective coatings or ARCs have been proposed by many researchers as a solution to this problem. By reducing the reflection losses, the efficiency of many devices like windows, solar panels and optical lenses can be increased.⁶¹⁻⁶³ The method comprises of placing layers of alternating high and low R.I. materials like $\text{TiO}_2/\text{SiO}_2$ or $\text{Nb}_2\text{O}_5/\text{MgF}_2$ on top of the substrate.⁶⁴⁻⁶⁵ The resulting final reflection is a combination of destructive interference occurring at various interfaces and hence the total loss is reduced. ARCs can be single layered with just one layer each of high and low R.I. materials or multi layered with the single layer structure repeated more than two times.⁶⁶⁻⁶⁷ Although there hasn't been much direct work on use of ARCs in SPR sensing, some works have still been done on utilization of ARCs in plasmonic devices. Ho et al. improved the plasmonic efficiency of silicon solar cells by using ARC of SiO_2 .⁶⁸ Gupta et al. used gold coated Cicada wings for plasmonic enhancement of fluorescence from upconversion nanoparticles.⁶⁹ Thus, taking inspiration from the effect of ARC seen in many optical devices, we make use of it in our simulation work where we examine how it can help in enhancing the sensitivity of SPR sensors.

3.5 Simulation of SARS-CoV-2 detection

COVID-19 hit the entire world in 2020 and all personal and professional aspects of every common man were badly affected. There have been many works where researchers utilized plasmonic-based detection method. Lautner et al. detected plant virus coat proteins using Deoxyribonucleic Acid (DNA) aptamers with the help of IBIS iSPR commercial sensing machine.⁷⁰ Chang et al. made a custom-made intensity modulated (IM) SPR for detecting Avian Influenza A H7N9 virus.⁷¹ Shi et al. could detect multiple respiratory viruses like Influenza A, Influenza B, H1N1, Adenovirus and SARS CoV using oligonucleotide probes on a PlexArray HT system manufactured by Plexera Bioscience.⁷² Bai et al. detected Avian Influenza Virus H1N1 using the Spreeta SPR detector by using DNA aptamers as the capturing molecule.⁷³ Vaisocherova et al. detected Anti-EBNA with a custom-made wavelength division multiplexing (WDM)-SPR system.⁷⁴ Additionally, there have been many examples where researchers have used either commercial SPR machines or have designed their own plasmonic-based system for detecting cowpea mosaic virus⁷⁵, human immunodeficiency virus (HIV)-1⁷⁶, hBV⁷⁷, Avian Leucosis Virus⁷⁸ and Influenza virus⁷⁹. Thus, our inspiration to design a plasmonic scheme and perform a simulative study to detect the COVID-19 virus has been derived from these works and in chapter 6 we look into the simulation that we performed in details.

Au nanoparticles have been used for many decades for enhancing the sensing properties of not only plasmonic sensors but also other sensors that work on different techniques. Lepinay et al. designed a tilted fiber Bragg grating (TFBG) optical fiber sensor that used Au nanocages (Au NCs)/Au nanospheres (Au NSs) for detection of various target proteins.⁸⁰ With the help of Au NPs, the researchers were able to lower the detection limit from the nano-molar range to the pico-molar range. Additionally, Le et al. used Au NSs that were stabilized with acetylated chitosan, and that showed plasmonic properties, for the detection of melamine, bacteria and uric acid.⁸¹ Moreover, many SPR-based schemes have been used in the past that have incorporated Au NPs for the detection of DNA hybridization⁸², oligonucleotide and p53 cDNA determination⁸³ and interaction assay of Carbamate-Acetylcholinesterase⁸⁴. Thus, we believe that Au NRs can be highly effective in enhancing the sensitivity and lowering the detection limit of conventional SPR scheme. Additionally, since NRs have a higher surface volume as compared to NSs, they can bind to more antibodies and hence it will lower the concentration of NR required. Thus, taking our simulation work further we designed an enhanced plasmonic detection scheme using Au NRs and in chapter 7 we discuss about the immunosensing system that we proposed.

In response to the evolving COVID-19 pandemic, many researchers have even come up with new plasmonic methods of detecting the SARS-CoV-2 virus. Cheong et al. devised a hybrid portable device that integrated reverse transcription, fast thermocycling using plasmonic heating of magneto-plasmonic NPs and in situ fluorescence detection after magnetic clearance of the NPs.⁸⁵ The device could detect the SARS-CoV-2 ribonucleic acid (RNA) in 17 minutes as compared to the standard 1-2 hour time using quantitative polymerase chain reaction with reverse transcription (RT-qPCR). The device could clearly differentiate between the COVID-19 patients and the healthy controls and the result had good accordance

with the standard RT-qPCR with the added advantage of quickness and portability. Ahmadvand et al. devised a functionalized tetrahertz plasmonic metasensor using Au NRs based on toroidal electrodynamics concept.⁸⁶ The sensor measures the resonance shifts due to the excitation of the toroidal dipole mode plasmons on the quasi-infinite metasurface. The detection process was rapid and precise with a low detection limit of 4.2 fM. Moitra et al. designed a naked-eye SARS-CoV-2 detector using a colorimetric assay based on Au NPs capped with thiol-modified antisense oligonucleotides (ASOs) specific to the N-gene (nucleocapsid phosphoprotein) of SARS-CoV-2.⁸⁷ The detection limit was 0.18 ng μ L⁻¹ and the detection process was relatively simple without the need of any sophisticated instruments. Additionally, Qiu et al. devised a hybrid approach to detect the SARS-CoV-2 virus using the concept of thermoplasmonic-assisted dual-mode transducing (TP-DMT) that uses amplification-free-based viral RNA detection and an amplification-based cyclic fluorescence probe cleavage (CFPC) detection.⁸⁸ Thus, we see that even in the uncertain times of the current pandemic, many research groups have come up with portable plasmonics-based POC technology for fast and accurate detection of the COVID-19. Many of these schemes can be extremely useful in remote areas of under-developed and developing nations that don't have access to world-class medicine facilities.

In chapters 6 and 7, we perform simulation work and design a plasmonic scheme that can be used for the detection of SARS-CoV-2 virus.

3.6 Biomolecular plasmonic detection using 2D materials

Most of the few earlier works that have used TMDC materials for plasmonic sensing have utilized non-prism based customized configurations like optical fiber with wavelength interrogation technique. 2D materials like MoS₂, Antimonene and graphene have been used for the detection of BSA, micro Ribonucleic Acid (miRNA), para-aminobenzoic acid (PABA) and Escherichia Coli (E.Coli). Kaushik et al. designed a fiber optic-based SPR sensor for detecting BSA.³⁸ The detection limit was 0.29 μ g/ml. However, this was done using a customized setup that used wavelength interrogation technique for measuring the change in resonance wavelength. Kumari et al. manufactured a Au nanoparticle (NP) based plasmonic sensor for detecting BSA that had an LOD of 90 pg/mm².⁸⁹ Xue et al. designed a SPR sensor based on Antimonene that could detect miRNA-21 and miRNA-155 having concentrations as low as 10 aM.³⁷ In another application, for detecting *E. Coli*, Kaushik et al. used MoS₂ nanosheets to design a fiber-optic type SPR sensor with an LOD of 94 CFU/mL.³⁶ Yu et al. used MoS₂-graphene nanostructure for SPR-based detection of Glucose that had an LOD of about 0.1 mg/dL.⁹⁰

Hence, in chapters 8 and 9, we use commercial SPR machine Biacore 3000 and perform basic bio-molecular testing using various 2D material coated gold chips like graphene, MoS₂ and WS₂. In chapter 10, using a customized setup we design a Goos-Hanchen shift-based plasmonic scheme with the help of graphene coated Au chips.

3.7 Conclusion

In this chapter, we saw some previous research works on sensitivity enhancement of SPR sensors using intermediate layers of nanomaterials. We also discussed about some works done by researchers on virological sensing. Additionally, we also saw how Au NPs were effectively utilized to enhance the sensitivity and also looked into some efforts made in the direction of designing SPR sensors based on 2D materials.

Chapter 4

SPR sensing scheme using titania and silica

Abstract—Surface Plasmon Resonance (SPR) based biosensors have been around in the scientific community for more than two decades and these have facilitated enormous improvement in the detection of complex biomolecules. Since, sensitivity of these sensors directly affects the ease of detection, our work here focuses on using dielectric nanosheets of titania and silica to enhance the sensitivity. The anti-reflective property of these dielectric materials promotes greater interaction between the incident light and the plasmonic metal, resulting in generation of more surface plasmons that promotes greater sensitivity. With 40 nm of gold, 3 layers of titania (9 nm) and a layer of silica (3 nm), our simulation results predict a sensitivity of 214 deg/RIU at 532 nm, which is a major improvement when compared to other sensor structures.

4.1 Fresnel's Reflection Laws and the Transfer Matrix Method (TMM)

Fresnel's reflection laws are useful while studying light interaction between multi-layered materials. To predict the sensitivity of multi-layered SPR structures, we make use of Fresnel's equations and the Transfer Matrix Method (TMM). The equations 4.1.1 – 4.1.7 show the respective mathematical relationships between various variables.¹⁴

$$M = \prod_{k=2}^{N-1} M_k \quad (4.1.1);$$

$$M_k = \begin{bmatrix} \cos \beta_k & \frac{-i \sin \beta_k}{q_k} \\ -i q_k \sin \beta_k & \cos \beta_k \end{bmatrix} \quad (4.1.2);$$

$$q_k = \frac{\sqrt{\varepsilon_k - n_1^2 \sin^2 \theta_1}}{\varepsilon_k} \quad (4.1.3);$$

$$\beta_k = \frac{2\pi d_k}{\lambda} \sqrt{\varepsilon_k - n_1^2 \sin^2 \theta_1} \quad (4.1.4);$$

$$r_p = \frac{(M_{11} + M_{12} q_N) q_1 - (M_{21} + M_{22} q_N)}{(M_{11} + M_{12} q_N) q_1 + (M_{21} + M_{22} q_N)} \quad (4.1.5);$$

$$R_p = |r_p|^2 \quad (4.1.6);$$

$$S = \frac{d\theta_{SPR}}{dn_{bio}} \quad (4.1.7),$$

where N , is the total number of layers, λ is the wavelength of the incident light, n_k and ε_k denote the R.I. and dielectric constant of the k -th layer, respectively, which are interdependent according to the relation $\varepsilon_k = (n_k)^2$, d_k is the thickness of each layer and θ_1 is the angle of the incident light wave, R_p is the reflectivity, θ_{SPR} is the angle of minimum reflectivity, n_{bio} is the R.I. of the analyte layer that contains the bio-molecules and S is the angular sensitivity, M is the characteristic matrix of the N -layer model and it is the main matrix involved in the Transfer matrix Method (TMM). For computing sensitivity, we observe the change in SPR angle (θ_{SPR}) for a minute change in R.I. ($\Delta n_{bio} = 0.005$).

Figure 4.1 Structure of the TiO₂-SiO₂ based SPR sensor for biomolecular detection.¹⁴

4.2 SPR sensing scheme using titania and silica

4.2.1 Sensor Structure

Titania (TiO₂) is a naturally occurring oxide of Titanium with many commercial uses. TiO₂ nano-crystals (NC) can show SPR effect and are thus a suitable material for optical devices. In addition, semiconductor-metal nano composites made from TiO₂ and metals like gold, silver and platinum can show localized SPR effect when illuminated by a suitable frequency light. Silica (SiO₂) is again a naturally occurring mineral that is abundantly found in the Earth's crust. There are numerous sensing applications of SiO₂ that utilize its optical properties like humidity and pressure sensing. SiO₂ coated plasmonic metals have given enormous contribution to diverse fields of sensing, bio-medicine, photocatalysis and photovoltaics. SiO₂ coated with gold plasmonic nanostructures have revealed about 50% gain in optical sensitivity. Biocompatibility plays an important role while choosing a suitable nano material for biological sensors since it can have an effect on the sensitivity. SiO₂ based nanomaterials have been extensively used in drug delivery and even in many human implants. Experimental studies have confirmed the utility of SiO₂ beads, embedded in glass microchips, in capturing DNA molecules from biological samples. Thus, our proposed sensor based on SiO₂ can be quite effective in capturing bio-molecules.

The structure of the sensor is shown in Figure 4.1. Starting from the bottom most layer, SF10 prism forms the base of the entire system which is coated by a thin metallic layer of gold film. N-layered TiO₂ (each layer of 3 nm) forms the third layer and it is then followed by a single 3 nm or 6 nm layer of SiO₂. We consider de-ionized (D.I.) water as the topmost analyte layer. Hence, the ARC sensor consists of five different materials: SF-10 prism, Au, TiO₂, SiO₂ and the analyte D.I. water. The thickness of SiO₂ is varied in the simulation, it is considered to be fixed either 3 nm or 6 nm. With other parameters varied like the thickness of Au, the total number of layers of 3 nm TiO₂ is evaluated. Hence, a N-layered TiO₂ structure has a thickness of 3N nm.

Figure 4.2 Basic SPR reflectivity curve at various incident light wavelengths of 532 nm, 600 nm, 633 nm and 785 nm.¹⁴

4.2.2 Modelling and Simulation Results

With the help of simulation we can arrive at the optimized condition that can give us best sensitivity results. For the optimization problem, we analyze for numerous configurations with varying thicknesses of the multiple layers involved in the structure. Also, we repeat the whole procedure at varying wavelengths. The different analysis wavelengths are $\lambda=532$ nm (green), 600 nm (orangish red), 633 nm (red), 785 nm (near infra-red (NIR)), 980 nm (NIR) and 1064 nm (NIR). Figure 4.2 highlights the basic SPR curve at different wavelengths. The dip in reflectivity is due to plasmonic resonances that occurs at an incidence angle where the wave vector of the incoming light matches with that of the propagation constant of the surface plasmons.

We varied the thickness of the gold film from 30 to 50 nm in steps of 5 nm and considered two different thicknesses of the SiO_2 layer, 3 nm and 6 nm. In totality, for every wavelength we had 10 experimental cases with different combinations of thicknesses of the Au film and the SiO_2 layer. At every combination, we varied the number of TiO_2 layers from 0 to 20 and observed the effect on SPR minimum reflectivity and sensitivity. At every wavelength, we selected the best combination that gave us the maximum sensitivity and upon further analyzing the entire data set at different wavelengths, we chose the best-optimized case comprising of the wavelength of light used, Au and SiO_2 thicknesses and the number of intermediate TiO_2 layers.

Table 4.1 briefly summarizes the simulation results and highlights the optimized condition, which occurs at a wavelength of 532 nm, Au thickness of 40 nm, SiO_2 thickness of 3 nm and three layers TiO_2 (9 nm). Elaborating slightly on the contents of the table, the sensitivity is dependent on multiple variables like wavelength, thickness and R.I. of each layer. In this table, the results at wavelengths of 532 nm, 600 nm, 633 nm and 785 nm have been summarized. The table should be read in a column wise format. Thus, considering column 2, the sensitivity of 214 deg/RIU is obtained at 532 nm, 40 nm Au, 3 nm SiO_2 and 3 layers or 9 nm of TiO_2 . Additionally, the minimum reflectivities and initial and final SPR angles have also been provided. Similarly, considering the last column, the sensitivity of 172 deg/RIU is obtained at 785 nm, 45 nm Au, 6 nm SiO_2 and 10 layers or 30 nm of TiO_2 .

Figure 4.3 (a)-(r) show the variation of minimum reflectivity and sensitivity with increasing number of TiO_2 layers for wavelengths ranging from visible to NIR region of the EM spectrum.

Table 4.1. Summary of the simulation results at various wavelengths ($\Delta n_{bio} = 0.005$)

Wavelength (in nm)	532		600		633		785	
Au thickness (in nm)	40	40	40	35	35	35	45	45
SiO ₂ thickness (in nm)	3	6	3	6	3	6	3	6
Number of TiO ₂ layers	3	3	5	5	6	6	10	10
Minimum Reflectivity R_{min} (in a.u.)	7.10×10^{-3}	1.88×10^{-5}	3.17×10^{-5}	8.60×10^{-2}	1.12×10^{-1}	7.68×10^{-2}	1.30×10^{-3}	7.10×10^{-3}
Initial SPR Angle (in deg)	78.89	80.82	82.47	81.64	81.28	82.66	81.08	82.10
Final SPR Angle (in deg)	79.96	81.85	83.43	82.58	82.21	83.56	81.99	82.96
Sensitivity (in deg/RIU)	214	206	192	188	186	180	182	172
FWHM (in deg)	14.40	14.44	13.93	13.99	13.65	13.58	12.72	12.49

From the reflectivity and sensitivity curves, we observe that the minimum reflectivity drops as we increase the TiO₂ layers but only till a limit, beyond which it again starts to increase. This can be explained by the fact that when the number of TiO₂ layers are initially increased, the incident light photons are mostly absorbed and transmitted rather than being reflected, resulting in a drop in reflectivity. However, further increase results in energy loss of the incident photons and it is not sufficient to excite enough plasmons. Similarly, for the same reasons, we observe an initial increase in sensitivity with enhanced number of TiO₂ layers and upon further addition, the sensitivity starts dropping, finally reaching zero at higher number of layers. We observe minima in the reflectivity curves and maxima in the sensitivity curves. This corresponds to the point or the number of layers of TiO₂ where there is maximum coupling between the incident photons and the free electrons on the metal surface resulting in the greatest number of plasmons being produced. The sudden dip in the reflectivity is due to the optimal condition occurring where the light-matter interaction is at its peak giving rise to maximum plasmons. The sensitivity curves peaks at a point and again this is the optimal condition. At lower number of layers of TiO₂, the sensitivity keeps increasing showing that additional layers help in reducing reflective losses of the photons and thus more photons interact with the free electrons producing more plasmons. Beyond the optimal condition or the maximum sensitivity point, the additional layers start to impede the light-matter interaction as it gives rise to optical losses. In many curves of sensitivity variation we observe a second peak at higher layers. The sensitivity curve here is a one-to-one variation and thus the sensitivity value at a particular point is a cumulative outcome of the input conditions: the combined effect of refractive indices and thicknesses of all layers. Although it is true that the general nature of the curve should be as follows: increasing sensitivity at lower layers of TiO₂, followed by a peak sensitivity value and then decreasing values of sensitivity beyond this peak. However, in the decreasing part of the curve, it is still possible to have small local rises and not a completely decaying trend as the sensitivity is a cumulative effect of the input conditions as described by the mathematical equations.

Many earlier reported works use a red light source (633 nm He-Ne laser). However, as shown here we can get an increment of 11.45% in the sensitivity if we operate at a lower wavelength of 532 nm.

Figure 4.4 shows the variation of change in SPR angle $\Delta\theta_{SPR}$ with respect to a change in R.I. of the analyte layer Δn_{bio} . We vary Δn_{bio} from 0.0000 to 0.01 in steps of 0.00005. We see that with increasing number of TiO₂ layers, the slope of the curve increases, indicating a greater change in resonance angle and thus a higher sensitivity. We observe this trend till N=3 layers of TiO₂ beyond which the slope falls below that of the basic Kretschmann configuration. This is due to the fact that greater number of TiO₂ layers adds up to additional losses causing less energy being transferred to the surface free electrons that can correspondingly lead to lesser oscillations and lesser production of plasmons.

Figure 4.3. Plots of variation of minimum reflectivity and sensitivity with increasing number of TiO_2 layers at wavelengths of 532 nm, 600 nm, 633 nm, 785 nm, 980 nm and 1064 nm and for various Au thicknesses of 30 nm, 35 nm, 40 nm, 45 nm and 50 nm.¹⁴

Figure 4.4. Variation of change in SPR angle with change in R.I. of analyte layer at 532 nm for 40 nm Au and other configurations with added layers of TiO₂ and SiO₂.¹⁴

Using Comsol Multiphysics 5.1, we simulate the electric field distribution for several models namely: the standard Kretschmann configuration and the modified configurations with 1L SiO₂, 1L TiO₂ + 1L SiO₂, 2L TiO₂ + 1L SiO₂, 3L TiO₂ + 1L SiO₂, 4L TiO₂ + 1L SiO₂ and 5L TiO₂ + 1L SiO₂.

Figure 4.5 shows the electric field distribution of the evanescent component E_y across various layers. The field propagates through the gold film and the intermediate TiO₂ and SiO₂ layers into the analyte layer only upto several nanometers of about 200 nm and then quickly vanishes after that. The electric field hot spot at the surface can be clearly seen where the field is at its maximum.

Figure 4.6 shows the variation of the normal electric field for different cases. The penetration depth of this field, which is defined as the depth at which the field is $1/e$ of its maximum value, is 89 nm. When we compare the various scenarios analyzed here, we see a huge enhancement in the field in the case of our proposed sensor structure and this directly contributes to its enhanced sensitivity. There is a 1682.24% increase in the evanescent field in our proposed design (3L TiO₂ + 1L SiO₂ over 40 nm Au) as compared to the Kretschmann configuration. The increase in peak field strength at the optimal condition of 3L of TiO₂ is due to the maximum light-matter coupling that takes place when maximum energy transfer occurs and most surface free electrons are set into oscillations that form maximum number of surface plasmons.

4.3 Comparison with other sensor layouts

Table 4.2 gives a comparison of the sensitivities between various SPR schemes. The percentages indicated in the last column are enhancements in the sensitivity of our TiO₂-SiO₂ sensor.

4.4 Surface Plasmons and Non-Locality

In all our previous cases we have considered the thickness of the dielectric material adjacent to the metal to be greater than 3 nm.⁹¹ However, when we go below this dimension, the classical EM method cannot be utilized to compute the sensitivity. When the gap distance falls below such low values, quantum effect changes the plasmonic coupling in nanoparticles. At distances below 3 nm, the non-local effect plays a crucial role and it cannot be ignored. At this interparticle gap, the R.I. of the plasmonic metal can no longer be considered to be a bulk value. To address this limitation, we make use of a local analogue model

Figure 4.5. Electric Field (E_y) distribution across the optimized SPR sensor structure based on SF-10 prism with 3L TiO₂ and 1L SiO₂ over 40 nm Au at SPR angle of 80 deg.¹⁴

Figure 4.6. Normal Electric field (norm. E) variation across different layers of SPR structure at 532 nm for various different layouts.¹⁴

Table 4.2. Comparison of the sensitivities between the TiO₂-SiO₂ configuration and other layouts.

Sr. No.	Type of sensor	Angular Sensitivity (in deg/RIU)	Active materials/structure of the sensor	Nano-structure of the sensor	Enhancement of angular sensitivity of our proposed sensor
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1	Kretschmann configuration	76	SF10 prism-Au	2.45 times (145%)
2	Si-WSe ₂ ⁵⁷	141.40	SF10 prism-Au-Si-WSe ₂	1.36 times (36%)
3	Si-MoS ₂ ⁴⁹	125.44	SF10 prism-Au-Si-MoS ₂	1.53 times (53%)
4	Si-WS ₂ ⁵⁷	155.68	SF10 prism-Au-Si-WS ₂	1.23 times (23%)
5	Cr-Ag-graphene ⁴⁸	68.03	SF10 prism-Air Gap-Cr-Ag-graphene	2.73 times (173%)
6	ZnO-Au-graphene-MoS ₂ ⁵⁰	101.58	SF10 prism-ZnO-Au-MoS ₂ -graphene	1.83 times (83%)
7	Graphene-MoS ₂ ⁴⁶	108	SF10 prism-BK7 glass slide-Au-MoS ₂ -graphene	1.77 times (77%)
8	Au-graphene ⁵³	95	SF10 prism-Au-graphene	2.02 times (102%)
9	TiO ₂ -SiO ₂ -MoS ₂ ⁴⁷	84.09	SF11 prism-TiO ₂ -SiO ₂ -Au-MoS ₂	2.21 times (121%)

(LAM) and change the modelling of the non-local dielectric and metal combination.⁹²⁻⁹³ This is usually done by representing the non-local entity with a thin dielectric metal of size Δl and permittivity ϵ_s on top of a local metal. The shifting of the metal boundary is denoted by⁹²

$$\Delta l(\omega) = \frac{\epsilon_s \lambda(\omega) \epsilon_M(\omega)}{[\epsilon_M(\omega) + 1]} \quad (4.4.1);$$

$$\lambda(\omega) = \frac{\sqrt{\frac{3}{5}} V_F}{\sqrt{\frac{\omega_p^2}{\epsilon_M(\infty)} - \omega(\omega + i\gamma_{metal})}} \quad (4.4.2).$$

where

$\epsilon_M(\omega)$ = Permittivity of plasmonic metal

$\lambda(\omega)$ = Penetration depth of surface charges

V_F = Fermi velocity of the metal

ω_p = Bulk plasma frequency

γ_{metal} = Damping frequency of metal

To get an idea of the process, we consider a model where 3 nm SiO₂ is coated on 50 nm Au placed on SF10 prism and compute the parameters at 532 nm. We evaluated the sensitivity using the conventional method without considering the non-local effect and found it to be 86 deg/RIU. When we considered the non-local effect, we modified the system using equations 4.4.1 and 4.4.2 and converted the 50 nm Au into a local metal of size $(50 - \Delta l)$ nm surrounded by a dielectric layer of thickness Δl nm of permittivity ϵ_s which we take as 1. Thus, we have a five-layer system, layer 1 is the SF10 prism, layer 2 is Au of thickness $(50 - \Delta l)$ nm, layer 3 is dielectric layer Δl nm, layer 4 is 3 nm SiO₂ and the last layer is that of analyte D.I. water. The various parameters calculated using equations 4.4.1 and 4.4.2 are given below.

V_F = Fermi velocity of the Au nanolayer = 1.4×10^6 m/s

ω_p = Bulk plasma frequency of gold = 1.1203×10^{16} rad./s

γ_{metal} = Damping frequency of metal = 1.3685×10^{14} rad./s

$\Delta l(\omega)$ = Thickness of dielectric layer = 0.11478 nm

$\lambda(\omega)$ = Penetration depth of surface charges = 0.10204 nm

Using these parameters, a sensitivity value of 84 deg/RIU is obtained. Though there is not much change in the value, we observe a slight drop as compared to the scenario when we use the conventional model without any non-locality effect being taken into account.

If the interparticle gap distance in this model is increased to 5 nm, both the conventional and LAM methods give the same value of sensitivity 86 deg/RIU. Hence, we can say, that when the feature size is higher than 3 nm, we can ignore the non-locality effect and use the conventional method. However, below 3 nm, a change in the modelling method is needed as detailed here so that an accurate estimate of the sensitivity can be made.

4.5 Conclusion

Improved healthcare is the need of the day. SPR biosensors have gained increased usage in bio & medical applications, e.g., for improved healthcare, because of their several distinctive advantages of real-time and label-free detection. However, a continual improvement in the sensor structure that fosters the development of an amplified sensing mechanism, is always desired. In our work, we are able to realize higher sensitivity using anti-reflective materials of titania and silica. Nanostructured layers of these materials can effectively reduce the amount of reflected light by virtue of their anti-reflective properties. With diminished reflection, more photons from the incident light can interact with the plasmonic metal, leading to the excitation of more surface plasmons that ultimately results in increased sensitivity. Summarizing our results, we can achieve a sensitivity of 214 deg/RIU at 532 nm with 40 nm of Au, 3 layers TiO₂ (9 nm) and a monolayer of SiO₂ (3 nm).

Chapter 5

Detection sensitivity enhancement using anti-reflective coatings (ARCs)

Abstract— Surface plasmon resonance (SPR)-based biosensors are an integral and indispensable aspect of present-day pathology, owing to its efficient real-time and label-free detection capabilities. Enhancing the sensitivity of these sensors is of paramount importance as it indirectly affects the detection limits of biomolecules. In this simulation work, we make use of antireflective coatings (ARCs) (double and multi-layered), made up of nanomaterials with alternating high and low refractive indices (R.I.) ($\text{TiO}_2/\text{SiO}_2$, $\text{TiO}_2/\text{MgF}_2$, $\text{Nb}_2\text{O}_5/\text{SiO}_2$ and $\text{Nb}_2\text{O}_5/\text{MgF}_2$); to enhance the sensitivity of Si-Transition Metal Dichalcogenides (TMDCs)-based SPR sensors. To summarize the entire outcome, our simulation is based on an already existing model based on Si and TMDC and the sensitivity of the $\text{TiO}_2\text{-SiO}_2\text{-Au-Si-TMDC}$ configuration is predicted to be 284 deg/RIU at 633 nm of incident light wavelength which is a great improvement in sensitivity.

5.1 Theory of ARC effect

Anti-reflective coatings (ARCs) are nano-engineered structural modifications specifically designed to alter the optical light trapping capability of photonic devices. An abrupt change of refractive index from the surrounding medium to the substrate causes a high amount of reflection loss. Anti-reflective coatings of sub-wavelength dimensions (generally of the order of few to several hundreds of nanometers), with refractive indices in between that of the substrate and the environment, can bring down the reflectivity, leading to a major improvement in efficiency. Stacking intermediate layers results in destructive interference of the reflected waves emerging out from the boundaries of different layers, which lead to a net cancellation of the reflected light and thus a reduced reflectance. Double and multi-layered ARCs are more common than single layered ones as they provide a broadband reduction in reflectivity, generally over the entire visible spectrum of EM light wave. These are predominantly made up of nano layers of alternating high and low refractive index (R.I.) materials like $\text{TiO}_2/\text{Nb}_2\text{O}_5$ (high R.I.) and $\text{MgF}_2/\text{SiO}_2$ (low R.I.). TiO_2 and SiO_2 are the two most common nano materials used as an ARC and these have been extensively used for the enhancement of performance of photovoltaic cells. Additionally, Nb_2O_5 and MgF_2 films are recently explored materials that possess high transmittivity over the entire range of visible spectrum and thus these can be good additions to the family of ARCs. In the SPR arrangement, the anti-reflection coating allows more incident light to get trapped and thus more surface plasmons at the metal-dielectric interface are excited. Since most of the incident energy is transferred in exciting more surface plasmons, the reflected light has a lower reflectivity and hence greater sensitivity can be realized. Figure 5.1 shows some simulation results on ARCs made up of TiO_2 and SiO_2 . We evaluate the variation of reflectivity as the wavelength of the incident light varies in the visible spectrum. We observe that when we put intermediate layers of anti-reflective materials TiO_2 and SiO_2 in between air and Si substrate, there is a marked reduction in reflectivity. Six layers of anti-reflective materials consisting of alternating layers of TiO_2 and SiO_2 (i.e. 3 layers of double layered ARC) gives the minimum reflectivity as compared to other arrangements. Hence, with the help of simulation it is possible to find out the wavelength that gives minimum reflectivity for a particular combination of ARCs.

Figure 5.1. Reflectivity variation with respect to incident light wavelength of Si-substrate with different arrangements of ARCs at an incident angle of 30 degrees.⁹⁴

5.2 Sensor Structure

We used antireflective coatings (ARCs) (double and multi-layered), made up of nanomaterials with alternating high and low refractive indices (R.I.) ($\text{TiO}_2/\text{SiO}_2$, $\text{TiO}_2/\text{MgF}_2$, $\text{Nb}_2\text{O}_5/\text{SiO}_2$ and $\text{Nb}_2\text{O}_5/\text{MgF}_2$); to enhance the sensitivity of Si-Transition Metal Dichalcogenides (TMDCs)-based SPR sensors.

The sensor configuration is depicted in Figure 5.2. There are seven layers starting from the SF-10 prism at the bottom and ending with the analyte layer at the top. The anti-reflective coatings ($\text{TiO}_2\text{-SiO}_2$, $\text{TiO}_2\text{-MgF}_2$, $\text{Nb}_2\text{O}_5\text{-SiO}_2$ and $\text{Nb}_2\text{O}_5\text{-MgF}_2$) form the second and the third layers respectively. The plasmonic metal (Au, Ag, Cu and Al) forms the fourth layer followed by silicon (5th layer) and TMDC (WS_2 , MoS_2 , WSe_2 , and MoSe_2) (6th layer). The analyte (de-ionized (D.I.) water) forms the last layer.

5.3 Simulation Results

We performed the simulations at 633 nm for four ARC combinations $\text{TiO}_2\text{-SiO}_2$, $\text{TiO}_2\text{-MgF}_2$, $\text{Nb}_2\text{O}_5\text{-SiO}_2$ and $\text{Nb}_2\text{O}_5\text{-MgF}_2$, four TMDC materials WS_2 , MoS_2 , WSe_2 and MoSe_2 along with four plasmonic metals Au, Ag, Cu and Al and hence we have 64 different sensor arrangements. We start by optimizing the thickness of TiO_2 and SiO_2 layers. Figure 5.3 (a)-(d) shows the variation of minimum reflectivity and sensitivity as a function of thickness of TiO_2 and SiO_2 . For optimization of TiO_2 , SiO_2 is fixed at 20 nm, Au at 50 nm, Si at 5 nm, WS_2 at 0.8 nm, MoS_2 at 0.65 nm, WSe_2 at 0.7 nm and MoSe_2 at 0.7 nm. Similarly, for optimization of SiO_2 , TiO_2 is fixed at 20 nm, Au at 50 nm, Si at 5 nm, WS_2 at 0.8 nm, MoS_2 at 0.65 nm, WSe_2 at 0.7 nm and MoSe_2 at 0.7 nm. We observe that for TiO_2 , both the minimum reflectivity and sensitivity increase as a function of TiO_2 thickness and reach a peak value beyond which these values start to decline. Thus, for optimum thickness, we choose it at a value where the minimum reflectivity is relatively smaller and the sensitivity is relatively larger than at other values of thicknesses. And this value is 95 nm for TiO_2 . At this thickness, the sensitivity is maximum for all TMDC materials and the minimum

Figure 5.2. Structure of the ARC modified Si-TMDC based SPR sensor for biomolecular sensing.⁹⁴

reflectivity is relatively smaller as compared to other thicknesses. For SiO_2 , the trend is a bit different. The minimum reflectivity keeps on decreasing as a function of SiO_2 thickness and the sensitivity also follows an overall declining trend. Thus, it is not possible to choose a thickness corresponding to smallest minimum reflectivity and largest sensitivity both at the same time. Hence, we go for a compromise and choose a thickness in the intermediate range, where the minimum reflectivity value is small (though not smallest) and the sensitivity is large (though not largest). And this value is 20 nm for SiO_2 . We use the same thickness as arrived here for all other combinations of ARCs and plasmonic metals.

After arriving at the optimized thickness values, for every unique sensor arrangement (64 sensor configurations in total), we vary the number of TMDC layers (N_{layer}) to arrive at the best thickness value ($d_{\text{TMDC}} = N_{\text{layer}} \times t_{\text{TMDC}}$) that gives us the highest sensitivity. Figure 5.4 (a)-(d) shows the simulation results for plasmonic metal Au for some TMDCs and ARC combinations. We observe a drop in minimum reflectivity and an increase in sensitivity as we increase the TMDC layers. This can be attributed to the fact that increased thickness of the dielectric material (TMDC), results in greater light-matter coupling. Thus, more energy is transferred to the free electrons on the plasmonic metal and hence more surface plasmons are produced that oscillate with greater amplitude during resonance giving a higher sensitivity. But this is only upto a certain number of layers beyond which losses start to occur and thus less energy can be transferred from the incident light to the free electrons and hence reducing the plasmons produced.

Table 5.1 summarizes the outcome of the simulation results. Along with sensitivity, we also calculate Full-Width at Half Maximum (FWHM), Detection Accuracy (D.A.) and Quality Factor (Q). The DA or the signal-to-noise ratio (SNR) depends on how accurately and precisely, the sensor can detect changes in resonance angle or the change in refractive index of the sensing medium. It is defined as the ratio of change in resonance angle to the angular width of the SPR curve corresponding to 0.5 reflectance. Thus, the higher the DA or SNR, the better the sensor. This parameter is quite different from detection resolution or the limit of detection (LOD), which is the minimum concentration level of a chemical or a biomolecule that can be detected by the sensor. Thus, this is quite opposite to the DA or SNR. The lower the LOD, the better the sensor. With Au as the plasmonic metal and $\text{TiO}_2\text{-SiO}_2$ as the ARC and with 2 layers of WSe_2 , we get the maximum sensitivity of 284 deg/RIU at 633 nm of incident light and this is the desired layout and has been highlighted below in Table 5.1.

(a)

(b)

Figure 5.3 Minimum Reflectivity and Sensitivity variation as a function of thickness of TiO_2 and SiO_2 at 633 nm for constant thicknesses of 50 nm, 5 nm, 20 nm and 20 nm for Au, Si, TiO_2 and SiO_2 respectively and for different TMDC materials.⁹⁴

(a)

(b)

(c)

(d)

Figure 5.4 Minimum Reflectivity and Sensitivity variation as a function of number of TMDC layers at 633 nm for SPR sensor based on Au with different combinations of ARCs and for different TMDC materials.⁹⁴

Table 5.1. Summary of simulation results at 633 nm ($\Delta n_{\text{bio}} = 0.005$)

S. No.	Plasmonic metal	ARC	TMDC	Initial SPR Angle	Final SPR Angle	Change in resonance angle ($d\theta_{\text{SPR}}$)	Sensitivity ($S = \frac{d\theta_{\text{SPR}}}{dn_{\text{bio}}}$)	FWHM	Detection Accuracy ($\text{D.A.} = \frac{d\theta_{\text{SPR}}}{\text{FWHM}}$)	Quality Factor ($Q = \frac{S}{\text{FWHM}}$)
1	Au	TiO ₂ -SiO ₂	WSe ₂ (2L)	74.55	75.97	1.42	284	22.79	0.062	12.46
2	Ag	TiO ₂ -SiO ₂	WSe ₂ (5L)	74.87	75.82	0.95	190	20.29	0.046	9.36
3	Cu	TiO ₂ -SiO ₂	WSe ₂ (6L)	74.54	75.61	1.07	214	21.43	0.049	9.98
4	Al	TiO ₂ -SiO ₂	WS ₂ (7L)	73.93	75.15	1.22	244	24.89	0.049	9.80

Figure 5.5 shows the electric field (E_y) variation across the sensor structure for the four different ARCs. We can easily observe the exponentially decreasing nature of the field. We see an increment in the evanescent field (in comparison to the standard Kretschmann configuration) for the different ARCs - 430.21% for TiO₂-SiO₂, 488.58% for TiO₂-MgF₂, 579.00% for Nb₂O₅-SiO₂ and 509.96% for Nb₂O₅-MgF₂. This can be attributed to the more light-matter interaction due to the presence of ARCs which help in reducing the reflectivity and thus more light gets transmitted or absorbed through these layers so that maximum energy transfer takes place between the incident light and the electrons at the metal surface. Figure 5.6 depicts a pictorial representation of the electric field distribution across the sensor surface. The field strength is maximum at the plasmonic metal surface and it decreases exponentially as it traverses towards the sensing medium. The L_p values for the various configurations are: 107.3 nm for standard Au, 105.25 nm for TiO₂-SiO₂, 110.15 nm for TiO₂-MgF₂, 110.34 nm for Nb₂O₅-SiO₂ and 116.8 nm for Nb₂O₅-MgF₂. Since the analyte layer begins at 55.8 nm (50 nm Au + 5 nm Si + 0.8 nm WS₂) from the metal surface, the electric field is still considerably high at this depth. The E_y values at 55.8 nm for the different configurations are: 11398.98 V/m for standard Au, 56486.24 V/m for TiO₂-SiO₂, 63998.98 V/m for TiO₂-MgF₂, 73777.59 V/m for Nb₂O₅-SiO₂ and 68569.97 V/m for Nb₂O₅-MgF₂. Therefore, the electric field values at the TMDC and sensing medium interface are higher for the various ARC configurations as compared to the standard Au.

5.4 Multi-layered ARC

In order to evaluate the efficacy of multi layered ARCs in further enhancing the sensitivity, we take TiO₂ and SiO₂ as the anti-reflective materials and assess its effect on sensitivity when they are placed suitably in the form of multiple alternating layers. We divide the available thicknesses of TiO₂ and SiO₂ into one-half, one-third and quarter widths to obtain alternate layers of high and low R.I. materials. For four layers of ARC, we use 47.5 nm of TiO₂ followed by 10 nm of SiO₂ and repeat the same. For six layers, we use 32 nm of TiO₂, 7 nm of SiO₂, 32 nm of TiO₂, 7 nm of SiO₂, 31 nm of TiO₂ and 6 nm of SiO₂. For eight layers, we use 23.75 nm of TiO₂ and 5 nm of SiO₂ and repeat it thrice. information depicts variation of sensitivity of multi-layered ARC as a function of number of WS₂ layers. Table 5.2 shows the simulation results for multi layered ARCs. There is a significant improvement in sensitivity (in comparison to double layered ARC) for Ag, Cu and Al. However, for Au, multi-layered ARC results in a diminishing sensitivity.

5.5 Comparison of sensitivity

There have been numerous configurations for SPR sensing that have been proposed before. Our model here makes use of a previously designed TMDC configuration and proposes further sensitivity enhancement in the defined TMDC structure using ARCs.⁵⁷ Thus, our basic premise here is to show the effectiveness of ARC in further elevating the sensitivity of pre-defined SPR structures. Biological sensing using SPR technique is quite common nowadays and it has some of its own distinctive advantages. Recently, several works have been reported that use hydrophobic interactions between the TMDC material (predominantly MoS₂) and the ligand antibody to detect the corresponding antigen.³⁶⁻³⁸ Thus, these MoS₂ based SPR sensors can provide easier and faster detection methods without the need of

Figure 5.5. Evanescent field (E_y) variation as a function of distance from the gold surface for SPR sensor using Au, Si and WS₂ with different combinations of ARCs at 633 nm.⁹⁴

Figure 5.6. Variation of electric field distribution across SPR sensor structure for different double-layered ARCs (a) TiO₂ – SiO₂ (b) TiO₂ – MgF₂ based on SF-10 prism, Au, Si and WS₂.⁹⁴

Table 5.2. Summary of simulation results for multi-layered ARC ($\lambda = 633$ nm and $\Delta n_{\text{bio}} = 0.005$)

Plasmonic Metal	Number of layers of ARC $\text{TiO}_2 - \text{SiO}_2$	TMDC material	Sensitivity (deg/RIU)	Sensitivity Enhancement (in %)
Au	2	WSe_2 (2L)	284	-
	4		262	N.A.
	6		244	N.A.
	8		242	N.A.
Ag	2	WSe_2 (5L)	190	-
	4		218	14.74
	6		218	14.74
	8		214	12.63
Cu	2	WSe_2 (6L)	214	-
	4		234	9.34
	6		226	5.61
	8		222	3.74
Al	2	WS_2 (7L)	244	-
	4		274	12.30
	6		230	N.A.
	8		220	N.A.

complex functionalization methods required to capture ligand antibody molecules in case of conventional gold based SPR sensors. Thus, our proposed model can be more advantageous in this regard as compared to other earlier proposed designs.

Table 5.3 mentions some of these arrangements based on simulation works done by other researchers at incident light wavelength of 633 nm and also gives a comparison of the sensitivity enhancement provided by our model, which is based on the ARC modified Si-TMDC structure.

5.6 Two-dimensional conductivity of 2D materials

Plasmonic metals like gold and silver are the two most common conductors used in SPR sensing. However, as we shall see in the next section, these metals suffer from intrinsic losses that are sometimes completely unavoidable. Certain fabrication techniques like electron beam lithography (EBL) lead to increased surface roughness that further aggravate the situation and lead to scattering losses. Moreover, the electronic properties of the noble metals cannot be altered after their fabrication.

2D materials like graphene and TMDC have much lower ohmic losses as compared to noble metals and additionally the electronic properties are tunable by applying electric field and adding impurities. These 2D materials have given extremely good results in the terahertz and infrared spectrum of the EM wave with high confinement of the electric field and low loss. In case of graphene, its electronic property can be changed from semiconductor to conductor by applying voltage and it can be tuned to support the generation of SPP. This is generally done by varying the Fermi energy level.

The 2D conductivity can be derived in what is known as the Kubo formulation. There are several forms that are in use and have been computed by many researchers. All the forms more or less operate in a similar fashion with slight variations depending on the Fermi energy. However, the most accurate representation will be presented.⁹⁵

Table 5.3. Sensitivity comparison of the ARC modified Si-TMDC structure with other SPR sensing models

Our Predicted Sensitivity (TiO ₂ -SiO ₂ -Au-Si-WSe ₂) in deg/RIU at 633 nm				284
S. No.	Type of sensor	Angular Sensitivity (deg/RIU)	Active nano-materials in the sensor	Enhancement of angular sensitivity in our proposed sensor (in %)
1	Kretschmann configuration	74	Au	283.78
2	Si-WS ₂ , Si-MoS ₂ and Si-WSe ₂ ⁵⁷	147.88, 131.7 and 141.4	Au-Si-TMDC	92.05, 115.64 and 100.84
3	ZnO-graphene-TMDC ⁷⁸	101.58	ZnO-Au-MoS ₂ -graphene	179.58
4	ZnO-graphene ⁵¹	187.43	ZnO-Au-graphene	51.52
5	TiO ₂ -SiO ₂ -MoS ₂ ⁴⁷	84.09	TiO ₂ -SiO ₂ -Au-MoS ₂	237.73
7	MoS ₂ -graphene-Al ⁵³	190.83	MoS ₂ -Al-MoS ₂ -graphene	48.82
9	Bimetal-ZnO-graphene ⁹⁶	222	Au-graphene-Au-ZnO	27.92
10	Graphene-BaTiO ₃ ⁵⁵	257	Ag-BaTiO ₃ -graphene	10.50
11	SnSe allotropes ⁵⁶	178	Ag-SnSe	59.55
12	Graphene-Si ⁹⁷	134.6	Au-Si-graphene	110.99
13	Graphene-MoS ₂ ⁴⁶	108	Au-MoS ₂ -graphene	162.96

The 2D conductivity can be represented by the following formulas. It has two components: interband related to the generation and recombination of electron-hole pairs and intraband that becomes similar to the Drude model at T=0 K.⁹⁵

$$\sigma_g = \sigma'_g + i\sigma''_g = \sigma_{intra} + \sigma_{inter} \quad (5.6.1);$$

The 2D conductivity extracted from Kubo formulations is defined by the relation below.

$$\sigma_g(\omega, E_F, \Gamma, T) = \frac{ie^2(\omega - i2\Gamma)}{\pi\hbar^2} \left[\frac{1}{(\omega - i2\Gamma)^2} \int_0^\infty E \left(\frac{\partial f(E)}{\partial E} - \frac{\partial f(-E)}{\partial E} \right) dE - \int_0^\infty \frac{f(-E) - f(E)}{(\omega - i2\Gamma)^2 - 4\left(\frac{E}{\hbar}\right)^2} dE \right]. \quad (5.6.2);$$

e , ω , E_F , $\hbar$, T , E , $f(E)$ and Γ are the charge of electron, radiation frequency, Fermi energy level, reduced Plank's constant, temperature, energy, Fermi distribution function and dispersion rate. The first and second parts of the equation are related to the intraband and interband conductivities. The dispersion rate Γ is given in terms of the electron relaxation time τ .⁹⁵

$$\Gamma = \frac{1}{2\tau} \quad (5.6.3);$$

$$\tau = \frac{\mu E_f}{eV_f^2} \quad (5.6.4);$$

μ and V_f are the carrier mobility and Fermi velocity of the material.

The 2D conductivity upon solving the Kubo formulations can be given by:⁹⁵

$$\sigma_{intra} = \frac{ie^2 k_B T}{\pi \hbar^2 (\omega + i\tau^{-1})} \left[\frac{E_f}{k_B T} + 2 \ln \left(\exp \left(\frac{-E_f}{k_B T} \right) + 1 \right) \right] \quad (5.6.5);$$

$$\sigma_{inter} = \frac{ie^2}{4\pi \hbar} \ln \left(\frac{2|E_f| - (\omega + i\tau^{-1})\hbar}{2|E_f| + (\omega + i\tau^{-1})\hbar} \right) \quad (5.6.6);$$

The Fermi level can be defined by⁹⁵

$$E_f = \hbar V_f \sqrt{\pi n_s} \quad (5.6.7);$$

$$n_s = \frac{\varepsilon_d \varepsilon_o |V_b|}{ed} \quad (5.6.8);$$

$$V_b = V_g - V_{dirac} \quad (5.6.9);$$

n_s is the doping level in the material. V_b is the bias voltage, V_g is the voltage applied to the material and V_{dirac} is the offset voltage due to natural doping. d is the thickness of the dielectric material below the 2D layer and ε_d , ε_o are the relative permittivity of the dielectric material and permittivity of free space respectively. Hence, the Fermi level can control the conductivity that in turn can be varied by tuning the bias voltage that affects the doping of the material. The permittivity and refractive index of the 2D material can be given by⁹⁵

$$\varepsilon_g(k, \omega) = 1 + \frac{i\sigma_g(k, \omega)}{\omega \varepsilon_o t_g} \quad (5.6.10);$$

$$n_g = \sqrt{\varepsilon_g} \quad (5.6.11);$$

k is the wave vector and t_g is the thickness of the 2D material. The refractive index can be tuned by varying the Fermi energy. For successful realization of SPP, the 2D material must behave as a conductor and this can be done by tuning the Fermi energy. When the Fermi energy is greater than 0.5 eV, the imaginary part of the refractive index increases whereas the real part decreases at the same time and this results in a change of behavior from dielectric to conductor. Also, for large values of thickness, the transition occurs at higher values of Fermi energy.⁹⁵

5.7 Losses in Plasmonics

Noble metals like Au and Ag are the primary plasmonic materials that are used in sensors and other devices. However, these metals suffer from intrinsic ohmic losses that can reduce the energy coupling between the incident EM wave and the free electrons giving rise to less plasmonic density and thus lower sensitivity. Moreover, the propagation distance of the plasmons is reduced. Now, this can seriously affect the evanescent field zone as with reduced penetration depth, the sensing region diminishes and this affects the probability of the bio-molecular interactions getting detected. Additionally, special patterns of the nanometal can lead to smaller grains, rough surfaces and semi-continuous films that can all give rise to scattering losses.

A part of the optical loss is attributed to the confinement of incident light in sub-wavelength dimensions of the metal NP. Thus, such kind of dissipation loss is highly evident and is completely unavoidable at high frequency and low wavelength of operation. The most important feature of all metals is their negative real part of the dielectric constant. The plasmonic mode can be observed when the frequency is lower than the plasma frequency, when the real part of the dielectric constant becomes negative allowing sub-wavelength confinement of SPP for different metal-dielectric layouts. This can be understood from the energy conservation principle. Inside a dielectric cavity, the energy oscillates between the electric and magnetic fields. However, once the dimension of a resonator approaches and is smaller than the

Figure 5.7. Band structure depicting metal absorption.⁹⁸

wavelength, the magnetic field decreases and becomes very small. In this case, the energy balance can no longer be maintained. The magnetic field and the electric field here are analogous to kinetic and potential energies in terms of mechanical energy. When the dimension of the resonator is in the range of its wavelength, the balance between the kinetic and potential energies is disturbed and the mode of operation stops as whatever energy is coupled into the cavity in the first half of cycle, it is radiated back in the next half of cycle. However, when free electrons are present, they have kinetic energy and the potential energy in the electric field gets transformed to the kinetic energy of free electrons. But whenever energy is transferred into the kinetic motion of free carriers, it is inevitably dissipated with the dissipation rate γ_m .⁹⁸

The field of optics, electronic and plasmonics are separated from their respective modes of functioning. Optics is dominated by displacement current due to bound carriers with features size greater than the wavelength. Electronics is governed by conductive current of free carriers with mechanical dimensions lesser than the wavelength. Plasmonics is dominated by displacement current of free carriers with feature size lower than the wavelength. Both optics and electronics can function at low loss, but the plasmonic region is inherently lossy due to the intrinsic lossy nature of the metal.

The loss in plasmonic metals is related to the imaginary part of the dielectric constant $\epsilon_2 = \frac{Ne^2}{m\epsilon_0\omega^3}\gamma_m$ and is linked to the scattering of conduction electrons by defects, surface states and lattice vibrations. N is the density of states, e is the electronic charge, m is the mass of electron, ϵ_0 is the permittivity of free space, ω is the frequency and γ_m is the metal scattering rate. The best approach here could be to reduce the metal defects, but this does not reduce the lattice vibrations that can only be possible by lowering the temperatures, which is not always feasible. The implicit assumption of Drude Theory is that the metal scattering rate γ_m is not a function of frequency and if the material is a good conductor at lower frequencies, it is also a good plasmonic material. But this is not always true. The scattering rate is related to the frequency and is dependent on the intricacies of the band structure of the material.

Figure 5.7 shows the typical band structure of a noble metal. The electrical conductivity is mainly due to partially occupied conduction band formed by the hybridization of outer s and p shells of Au. The Fermi level here separates the occupied states filled with electrons from the unoccupied ones. The energy of the metal interacting with the EM wave of frequency ω can only be incremented in steps of $\hbar\omega$ and the loss can be described from the absorption of photons. The energy from the photon is used to transfer electrons from the filled states below the Fermi level to the unfilled states above this level. As the momenta of the two levels $\hbar k$ is different, additional scattering mechanisms like lattice vibrations (phonons), surface imperfections, impurities or additional electrons excited from the filled states below the Fermi level are required. As depicted in Figure 5.7a, for lower frequencies ω_1 , the absorption of a low energy photon requires simultaneous absorption of a phonon. Only when the frequency is small, the

absorption is limited to the DOS and the scattering states near the Fermi surface. When the frequency is increased to higher values ω_2 , more states are involved in absorption and it is no longer restricted to the filled states near the Fermi level. The entire conduction band is involved. As shown in Figure 5.7a, for higher frequencies, the absorption of a high energy photon can be led by either absorption or emission of a phonon. At higher frequencies, the d shell electrons that lie below the Fermi level and overlap with the s-p conduction band, start getting involved. Now at even higher frequencies, when the photon energy approaches the “interband edge” value $\hbar\omega_B$, which is 1.74 eV for Au, the electrons start their transition from the d-states to the unoccupied states. These interband transitions are direct and they do not require additional scattering as the momenta of the initial and final states are equal. The d-band is relatively narrow than the hybridized s-p band and has low DOS and thus at higher frequencies, the metals become strong absorbers increasing the loss further. At even higher frequencies ω_3 , the absorption enhances and additional interband transitions take place from the main conduction band to the higher unfilled states. The absorption that is dependent on the imaginary part of permittivity ϵ_2 , is found by many states at different energies and has mainly two components, the intraband due to the free electrons in the s-p band and the interband component due to direct transitions between the bands. The free-electron component is denoted by $\epsilon_{2,fe} = \frac{Ne^2}{m\epsilon_0\omega^3}\gamma_m(\omega)$. The difference is that the metal scattering rate is a function of frequency.

The interband component $\epsilon_{2,ib}$ is similar to the ϵ_2 of a semiconductor, very low at frequencies below ω_B and very high above this cut-off frequency as shown in Figure 5.7b. Thus, for lower losses it is desirable to operate the system at lower frequencies. But, even at lower frequencies, the intraband component gives rise to losses that are dependent on the states in the entire conduction band and not just limited to the states near to the Fermi surface. The real part of the permittivity ϵ_1 is responsible for the reflection and refraction of the EM wave. Because energy is not being transferred to the metal, the transitions for ϵ_1 are virtual transitions where the photon tries to push the electron to the higher state but does not have the energy for that. The electron ends up at the same level. The real part of permittivity is accurately represented by the Drude model.

Thus, we see that two major losses arrive here, one due to the sub-wavelength dimension of the metal nanoparticle and other due to the intrinsic scattering losses in the metal that arises due to the transitions involved in the band structure of the metal. All these losses limit the energy coupling between the photon and the free electrons such that the free electron oscillations become damped and the SPP have reduced propagation length. This reduces the available sensing region and makes the detection process difficult. Several approaches are being employed by researchers in order to minimize these intrinsic losses. These can be divided into three main categories: (1) Designing the shape and size of the plasmonic structures such that less fraction of energy is confined inside the metal (2) inserting gain elements into the plasmonic or metamaterial structures to compensate the losses in metals (3) considering other materials like highly doped semiconductors, intermetallics and graphene which have low density of states.⁹⁸ Many researchers have discovered new band structure engineered materials that have lower intrinsic losses. These band structured layered materials like TaS₂ and other TMDC materials have specially designed low density of electronic states such that the probability of scattering losses is extremely reduced.¹⁰⁰ The condition of ideal loss-less material arises when there are no states available for scattering in a particular frequency range. Such a scenario is possible only when partially filled bands are separated from all higher and lower lying bands by relatively high energy gaps. Lower dimensional layered materials with a high amount of under-coordinated atoms and weaker hybridization exhibit such band structures. Many 2D materials like TMDCs WS₂, MoS₂ and graphene exhibit such structures.

5.8 Intricacies in 2D material based plasmonic sensing

Although 2D materials have garnered huge interest among researchers in the bio-medical fraternity, several other complex features can actually affect the plasmonic performance of these materials and grain boundary is one of them. Specifically, grain boundaries (GBs) are imperfections in the lattice structure that can significantly alter the material properties and also its sensing performance. In a 2D crystal, disinclination pairs form dislocations and grain boundaries one-dimensional chains of aligned dislocations. In case of GB related issues of 2D materials, three major concerns are as follows:

1. Characterization of the detailed microstructure and the GB patterns in the 2D structure for which advanced imaging techniques like high resolution transmission electron microscopy (HRTEM) and scanning tunneling microscopy (STM) are needed.

2. Correlation between the synthesis method and the GB patterns developed on the 2D material. The chemical vapor deposition (CVD) method has been known to be the most versatile and economic method that can manipulate the quality and properties of the 2D material.
3. Understand the growth process and develop methods to produce high quality 2D material like properly tuning the GB density to alter the properties and match with the desired features.

GBs can play a critical role in 2D material performance as they can alter the properties. Talking about the electrical properties, GBs can reduce the conductivity and mobility of graphene and hence the conductive properties can get affected. Some researchers even found that point defects and surface impurities of CVD-developed graphene have little effect on mobility and that the GB was the main contributing factor. STM studies for measuring change of electrochemical potential of current through graphene revealed that there is a sudden change of potential and a corresponding decrease of electrical conductivity at the GB of graphene. Some researchers claim that the resistance at the GB is 1.4 times higher than that observed in the crystal region and that there is correlation between the GB resistance and the grain region size. In contrast to this, some researchers have found that the grain size or GB does not affect the conduction at all. For instance, a type of structure called sinuous grain boundaries (s-GBs) in graphene showed improved semiconducting behavior and mechanical strength at the GB.¹⁰¹⁻¹⁰⁴

The electronic structure of the TMDC materials can also be affected by the GB. In one study it was shown that the electron transport across the GB in MoS₂ is unchanged whereas transport along the GB direction is improved. In another study it was found that only tilt GBs have a significant impact on the mobility and transport characteristics of MoS₂ and the differences in tilt GBs are due to the congregation of point defects or other structural defects. Additionally, it was also found that GBs in MoS₂ with a tilt angle caused reduced mobility.

Coming to the chemical properties, it has been shown that a GB region is characterized by high chemical activity as compared to the adjoining lattice areas. In case of graphene, the presence of increased chemical reactivity at the GB can enhance the sensitivity of the sensor. Also, hanging bonds, topological defects or even reaction with H can regulate the magnetic properties of graphene. Thus, understanding the formation of GBs is essential so that they can be engineered by the growth process and the properties of the 2D material can be manipulated. Even in TMDCs, the GBs have enhanced catalytic activity as compared to its basal plane.

Moving to the thermal properties, it was found that the thermal transport is reduced across the graphene GBs. Some experimental studies have revealed that the thermal conductance through a secluded GB can be much lower than the theoretically derived values. Additionally, tilt GBs showed the lowest conductance when out-of-plane distortions were added on GBs that led to a reduction in the contribution towards the transmission from in-plane distortions. It was also found that tilt GBs induce high-frequency nondispersive phonon modes in the phonon spectrum of polycrystalline graphene that are strongly limited to the core of the GB as compared to the case of translational boundary system where such high-frequency localized modes do not occur.

The mechanical properties of the 2D materials also change at the GB. It was found that the presence of GB in graphene enhances its mechanical strength and that the atomically thin membrane at the GB can sustain huge amounts of strain. Some studies show that mechanical properties of large-angle GBs are similar to that of intrinsic graphene in contrast to the case where small-angle GBs show decreased strength. In another experiment where the elastic modulus and mechanical strength of GB-controlled CVD grown graphene films were measured, it was found that the graphene films became stronger by increasing the density of GBs. The GB also affects the mechanical properties of TMDC materials. However, some studies have shown opposing results. It was found that CVD-grown monolayer MoS₂ having cohesive GBs showed pronounced deterioration in terms of in-plane mechanical properties.

Thus, to conclude we see that a varied number of outcomes is possible that are contrasting in nature. For instance, although electrical and thermal properties of graphene is degraded in most cases because of the presence GB, the chemical and mechanical properties are greatly enhanced. Hence, properly tuning the CVD growth method becomes essential so that the GBs can be engineered to meet the desired properties and features. GB manipulation can mainly be done by the growth process or post-growth treatment. Many such research works have been undertaken where the growth methods have been fine tuned to properly engineer the GB so that the sensing capabilities of the 2D materials is least affected or in some cases even enhanced. Like, Liu et al. designed GB-rich polycrystalline monolayer WS₂ films for attomolar-level

sensing of Hg^{2+} ions and found that controllably-grown polycrystalline films of 2D layered materials with high-density GBs had abundant structural defects that acted as conducive sites for adsorption of ions and resulted in the formation of ultra-sensitive sensors.¹⁰⁵

Moving on to the next aspect of interlayer interaction between 2D materials, the topic of van der Waals (vdW) heterostructures and its properties needs to be talked about. These heterostructures are made up of vertical stacks of atomically thin 2D materials and the properties are dependent on the constituent 2D materials and the physical interaction between the layers. The huge family of 2D materials comprises of insulators, semiconductors, semimetals and superconductors made up of graphene, hexagonal Boron Nitride (h-BN), black Phosphorus (BP), topological insulators (Tis), TMDCs, MXenes etc. and all these materials show varied bandgap structures and enhanced extraordinary light-matter interactions due to which these get accepted as the most preferred material in plasmonic sensing, ultrafast photodetection, photovoltaics and other related fields. When these materials are stacked together as vdW heterostructures, the electrical and optical properties are modified and can give a whole new edge to the device application.¹⁰⁶⁻¹⁰⁸

These materials are generally prepared by the mechanical transfer method, chemical vapor deposition (CVD) growth method and solvothermal method. In recent years the research on ultrafast optical properties of vdW heterostructures has attracted a lot of attention. Many researchers have reported the evidence of ultrafast charge transfer in photoexcited vdW heterostructures and have found the charge transfer rate to be in the range of femtoseconds that is extremely useful for ultrafast photonics. The ultrafast response in the vdW heterostructure can be understood in terms of their band alignment. There are two types of alignment namely Type-I and Type-II. For type-I, the conduction band minimum (CBM) and valence band maximum (VBM) are in the same material and has a narrow band gap. The photoexcited electrons and holes in the material having a wider band gap can transfer to the material having a narrower band gap. However, the reverse is not possible and the photoexcited carriers in narrow band gap material cannot move to the other layer because of lower energy. In type-II alignment, the CBM and VBM are located in different materials and the photoexcited electrons and holes can separate rapidly due to ultrafast charge transfer.

In this aspect, TMDC/TMDC vdW heterostructures are widely studied. The band alignment in these materials is mainly of type-II and the electrons and holes stay in different materials after photoexcitation. Although the interlayer forces of attraction in these heterostructures is weak, the charge transfer process can take place efficiently. Many researchers have studied the photoluminescence (PL) spectra of the individual 2D materials and the vdW heterostructures made up of the individual materials where the quenched photoluminescence showed the evidence of efficient transfer of electrons and holes. They found the charge transfer times to be of the order of few femtoseconds in MoS_2/WS_2 , $\text{MoSe}_2/\text{MoS}_2$, $\text{WSe}_2/\text{MoS}_2$ and $\text{MoTe}_2/\text{MoS}_2$ heterostructures. Some theoretical investigations have also been carried out to explain the fast charge transfer rate in vdW heterostructures and the charge transfer evaluation of $\text{MoS}_2/\text{MoSe}_2$ showed that quantum coherence and donor-acceptor delocalization favor ultrafast charge transfer at the interface of the two materials.¹⁰⁹

Many such vdW heterostructures have also found useful application in the field of ultrafast infrared photonics. The presence of dangling-bond-free surface and weak vdW interlayer forces between the layered structures, random combinations of 2D materials can be chosen to be stacked in an arbitrary order to form varied types of vdW heterostructures. With the availability of multiple degrees of freedom like the material used in the layered structure, twisted angle of the component layers and others, the electronic properties of the heterostructure can be fine-tuned to meet the desired characteristics.

In this regard, many researchers have actually been able to engineer vdW heterostructures with enhanced electronic and optical properties. Aggoune et al. fabricated graphene and h-BN vdW heterostructure and found that by varying the stacking arrangement, the most stable parameters with shortest out-of-plane lattice parameters could be achieved that had where the electrostatic and Pauli repulsions between the layers was minimum.¹¹⁰ The heterostructure also showed good absorption over a broad range of frequency from the near-infrared (NIR) to the ultraviolet (UV) region. In another work, Tang et al. combined BP with monolayer MoS_2 and found that the heterostructure showed high photodetection sensitivity.¹¹¹ Due to the high light absorption in the visible range and reduced electron-hole recombination rate because of strong carrier confinement, the photoresponsivity of the BP/ MoS_2 heterostructure was much higher than pure BP.

Thus, when stacking multilayer 2D materials to form vdW heterostructures, the individual layers do not behave as intended since the interlayer interactions modify the structural, electronic and optical properties of the entire configuration. However, by varying the stacking arrangement, combination of 2D materials and other factors, the properties can be tuned to match the desired requirements of the sensor.

5.9 Conclusion

SPR based bio sensing can give tough competition to other technologies as these can help in the early detection of rare life-threatening diseases, when other conventional detection schemes fail to give reliable results. Hence, it is quite crucial to ensure high sensitivity along with good accuracy, for these sensors. In this work, we demonstrate that by making use double layered ARCs, we can allow more photons to get absorbed and transmitted through the sensing platform, thus allowing greater interaction between itself and the plasmonic metal that leads to enhanced surface plasmon generation, and finally a boost in the sensitivity. With the help of simulation based on the Fresnel's equations for multi-layer reflection, we present a detailed analysis using four kinds of TMDCs (WS_2 , MoS_2 , WSe_2 and MoSe_2), four combinations of ARCs (TiO_2 - SiO_2 , TiO_2 - MgF_2 , Nb_2O_5 - SiO_2 and Nb_2O_5 - MgF_2) and four different plasmonic metals (Au, Ag, Cu and Al). We obtain a sensitivity of 284 deg/RIU at 633 nm of incident light wavelength with the TiO_2 - SiO_2 -Au-Si-TMDC configuration. Additionally, we also evaluate the usefulness of multi layered ARCs in further improving the sensitivity, when compared to the double layered ARC arrangement.

Chapter 6

Plasmonic detection of human respiratory viruses

Abstract- The COVID-19 virus has been recently identified as a new species of virus that can cause severe infections like pneumonia. The sudden outbreak of this disease is being considered a pandemic. Given all this, it is essential to develop smart bio-sensors that can detect pathogens with minimum time delay. Surface Plasmon Resonance (SPR) bio-sensors make use of refractive index (RI) changes as the sensing parameter. In this work, based on actual data taken from previous experimental works done on plasmonic detection of viruses, we have performed a detailed simulation of the SPR scheme that can be used to detect the COVID-19 virus and have extrapolated the results from earlier schemes to predict some outcomes of our SPR model. Our results indicate that the conventional Kretschmann configuration can have a limit of detection (LOD) of 2×10^{-5} in terms of RI change and an average sensitivity of 122.4 degRIU-1 at a wavelength of 780 nm. Thus, by comparing and extrapolating experimental results from an earlier Avian Influenza Virus (AIV) SPR detection scheme, we show how the plasmonic model can be utilized in the preliminary design of virological sensors.

6.1 Introduction

The last day of the year 2019 saw the sudden outbreak of many cases of pneumonia in Wuhan, situated in the Hubei province of China that seemed to be caused by an unknown bug. Later, it was found out that the pathogen was a unique strain of a coronavirus that was never seen before and they named it as COVID-19. To date, the number of confirmed and suspected cases have been exponentially rising and there have been no signs of relief. Coronaviruses belong to the family of Coronaviridae and other Nidovirales and can majorly be seen in humans and mammals. The animal source of COVID-19 has not been identified yet, however it is believed that the first few cases had direct exposure to an animal source from a live animal market in China. The symptoms of this disease can range from mild cough, runny nose, sneezing and running fever to severe lung congestion and pneumonia. The virus can spread by coming into close contact with infected people by being exposed to their phlegm or cough droplets or by coming in contact with infected surfaces. Because of constant flow of people in and out of China, the situation has become pandemic and there have been several cases outside China. Thus, the WHO has announced this outbreak as a cause of global concern. Compared to other earlier serious coronavirus outbreaks in the past like Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012 and Severe Acute Respiratory Syndrome Coronavirus (SARS – CoV) in 2002, which had high mortality rates, the COVID-19 virus has a relatively lower death rate of 2 percent. However, the transmission rate of this particular newly found strain of virus is higher than the earlier viruses of the same family.

Acute respiratory diseases (ARD) are quite common and can affect children and adults of any age. Major viruses that have caused ARD include parainfluenza virus (PIV) type 1 (PIV1), PIV2, PIV3, PIV4, respiratory syncytial virus (RSV), influenza A and B viruses, bocavirus, adenovirus, rhinovirus, coronavirus (CoV), enterovirus, the newly discovered parvovirus types 4 and 5 and mimivirus. Rhinoviruses and CoVs have been affecting humans since the 1960s. However, until recently the medical fraternity never gave much attention to them since their impact was considered to be minor. CoVs can be fatal and in some cases they can cause severe lower respiratory tract infections (LRTI) and hence diagnostic testing to determine the pathogen becomes really important. Therefore, developing an efficient and reliable diagnostic system that can differentiate and distinguish among these pathogens is of paramount importance. Table 6.1 shows a list of several diagnostic methods that are used for detecting respiratory viruses and it also gives their drawbacks. The tests are mainly categorized into Serological Testing: Hemagglutination inhibition assay (HI), Complement fixation test and Enzyme immunoassays like Enzyme Linked Immunosorbent Assay (ELISA), Immunofluorescence : Direct fluorescence antibody (DFA) and Nucleic Acid Amplification Test (NAAT) : Reverse Transcription

Table 6.1. Diagnostic methods of respiratory viruses

S.No.	Genre of test	Current respiratory virus detection technique	Drawbacks	Signal measured
1.	Serological testing	Hemagglutination inhibition assay (HI) ²	1. Low sensitivity and specificity	Agglutination level observed under microscope.
2.		Complement fixation test	1. Low sensitivity 2. Time-consuming 3. Non-specific	Presence of cell lysis of sheep red blood cells observed under microscope.
3.		Enzyme immunoassays like Enzyme Linked Immunosorbent Assay (ELISA)	1. Expensive kits 2. Well-trained technicians required 3. False positive/negative results possible with mutated antigens	Absorbance values produced from the substrate-enzyme complex.
4.	Immunofluorescence	Direct fluorescence antibody (DFA) ²	1. Cross reactivity 2. Careful controls to ensure no false positives/negatives are present	Fluorescence observed under microscope
5.	Nucleic Acid Amplification Test (NAAT)	Reverse Transcription Polymer Chain Reaction (RT-PCR), qPCR ²	1. Time consuming 2. Trained analysts required 3. Systematic and careful collection, handling and transportation of specimen 4. Genetic variability of RNA can result in mismatches between primers and target sequences giving false negative results	Fluorescent signal from DNA binding dyes
6.		Nucleic acid sequence-based amplification (NASBA) ³	1. High quality RNA required 2. Maintenance of reaction temperatures up to 42 deg Celsius 3. Target RNA sequence should have 120-150 nucleotides for optimal amplification	Fluorescent signal from molecular beacons attached to RNA.
7.		Loop-mediated isothermal amplification (LAMP) ⁴	1. Proper design of primer 2. Less sensitive in case of complex samples like blood	Fluorescence values DNA binding dyes.

Table 6.2. Earlier works on SPR-based detection of viruses

S.No.	Name of virus	Capturing molecule	SPR Scheme	Detection Limit	Signal measured
1.	Plant virus coat proteins ¹⁹	DNA aptamers	IBIS iSPR (IBIS Technologies BV, Hengelo, The Netherlands)	250 nM	Angular modulation
2.	Avian Influenza A H7N9 virus ²⁰	H7-mAb	Custom made Intensity modulated (IM) – SPR	402 copies/mL	Voltage measured in mV
3.	Multiple Respiratory Viruses (Influenza A, Influenza B, H1N1, RSV, PIV 1,2,3, Adenovirus, SARS CoV) ²¹	Oligonucleotide probes	PlexArray HT system, Plexera Bioscience	Influ A – 5 nM Influ B – 1 nM PIV ₁ – 1 nM PIV ₂ – 2.5 nM PIV ₃ – 3.5 nM RSV – 3 nM ADV – 0.5 nM SARS – 2 nM H1N1 – 3 nM	Angular modulation
4.	Avian Influenza Virus H1N1 ²²	DNA aptamers	Spreeta SPR detector, Texas Instruments	0.128 HAU	Angular modulation
5.	Anti-EBNA ²³	BSA-EBNA	Custom made WDM-SPR	1 pM	Wavelength modulation
6.	Cowpea Mosaic Virus ²⁴	Single Chain Variable Fragment scFv molecules	Biacore X	12.5 µg/mL	Angular modulation
7.	HIV-1 ²⁵	Streptavidin-Biotin modified chips	Biacore 1000	16.6 µg/mL	Angular modulation
8.	hHBV ²⁶	Anti-HBV	Spreeta SPR, Texas Instruments	9.2 nM	Angular modulation
9.	Avian Leucosis Virus ²⁷	mAb ALV-J	Custom made SPR waveguide immunosensor	---	Wavelength modulation
10.	Influenza virus ²⁸	α2-3 Sia glycan and α2-6 Sia glycan	Biacore T100	3.125 nM	Angular modulation

Polymer Chain Reaction (RT-PCR), qPCR, Nucleic acid sequence-based amplification (NASBA) and Loop-mediated isothermal amplification (LAMP). Serological testing determines the presence of any antibodies that result because of the presence of the corresponding antigens obtained from being infected with the respective viruses. Immunofluorescence measures the fluorescence of a fluorescent dye that

attaches to the antibody. NAAT amplifies the nucleic acids Deoxyribonucleic Acid (DNA) or Ribonucleic Acid (RNA) and measures the fluorescent levels of dyes that attach to these amplified molecules.

The physics behind the concept of Surface Plasmon Resonance (SPR) came into existence in the 1980s. Surface Plasmons (SP) are formed as a result of interaction between electromagnetic (EM) light waves and nano-sized metals like gold (Au) or silver (Ag). Plasmon-based immunoassays have garnered a lot of attention in the field of bio-molecular sensing. SPR-based assays have several advantages that can overcome the drawbacks and limitations of other conventional methods of testing. These assays rely on refractive index (RI) changes for detection of bio-molecules and therefore they do not need any label. Since only small amount of sample sizes are required for testing, this technique can be very cost-effective. Additionally, commercial SPR machines can handle complex samples and hence it is not mandatory to have extremely pure high-quality samples if proper care is taken while sample injection through the microfluidic channels and the instrument is properly maintained by frequent regular clean-up of its internal components. Moreover, the results are repeatable and have high accuracy. In short, plasmonic detection technique offers label-free and real-time approach towards investigation of bio-molecular reactions. Table 6.2 shows some previously designed SPR schemes that have been used for detection of several viruses. It lists down the ligand molecules used for capturing the viruses, the specific SPR scheme, the limit of detection and the interrogation technique or the signal measured. There have been many SPR schemes where commercial sensing equipments have been used for detecting respiratory viruses like PlexArray HT system used for detecting viruses like Influenza A, Influenza B, H1N1, RSV and many more, Spreeta SPR detector for detecting Avian Influenza Virus (AIV) and Biacore T100 for detecting Influenza Virus.

6.2 Sensor Structure

The basic SPR sensing scheme consists of 50 nm Au coated on a high RI glass prism. The reason behind choosing 50 nm Au is that the basic Kretschmann arrangement uses standard 50 nm Au and the commercial sensors available in the market too make use of this standard arrangement. Hence, we have three layers. BK7 prism forms the first layer. The plasmonic metal Au is the second layer and the analyte D.I. water acts as the third layer. Commercial sensors generally use buffer solutions as the analyte. For instance, Biacore 3000 uses Hepes Buffered Saline (HBS) as the analyte. Since the RI of HBS is not very different from that of D.I. water, we believe that changing the analyte to running buffer solution will not cause a significant change in the simulation results. The sensing scheme has been depicted in Figure 6.1. The Au layer is above the BK7 prism. It is followed by functionalizing molecules that can capture the antibodies followed by the analyte. To effectively capture the target pathogen molecule under test, it is essential to functionalize the Au surface using ligands like antibodies or aptamers. We also perform additional simulation on graphene modified SPR scheme. In this case, we add N_{gh} layers of 0.34 nm graphene between Au and the analyte. Hence, there are total four layers in this modified layout where graphene is now the third layer and the analyte forms the fourth layer.

We perform the simulations at $\lambda = 690$ nm, 780 nm and 830 nm with the help of equations 4.1.1 to 4.1.7. We vary the RI of the analyte layer n_{bio} from 1×10^{-5} to 1.1×10^{-3} in steps of 1×10^{-6} and calculate the sensitivity. For the graphene modified structure, we first optimize N_{gh} and arrive at a configuration that gives us maximum sensitivity. For calculating the sensitivity here, we take $\Delta n_{bio} = 0.005$. Next, for the optimized scheme, we vary the RI of the analyte layer from 5×10^{-6} to 1.1×10^{-3} in steps of 1×10^{-6} and calculate the sensitivity.

Figure 6.1. Schematic of the SPR setup for virological sensing.¹¹²

6.3 Results and Discussion

Figure 6.2 gives the variation curve of sensitivity versus change in RI for the three wavelengths. Δn_{bio} has been varied from $5E-6$ and $1.1E-3$ and the respective sensitivities have been calculated. The minimum detection limits in terms of change in RI are: $690 \text{ nm} - 5.1E-5$, $780 \text{ nm} - 2E-5$ and $830 \text{ nm} - 6.8E-5$. The step change in R.I. has been considered is $1E-06$. There are no discontinuities in the curves. A particular change in R.I. has just one unique sensitivity value, it is a one-to-one relation. However, the sudden peaks and jumps in sensitivity is completely mathematical as the sensitivity is a complex function of change in R.I. For instance, if a three-layer media is considered, the sensitivity can be represented as shown in the equation below. Hence such variations can be attributed to the nature of the inverse sine curve. At first glance it seems that there are discontinuities because of the compactness of the scale, there are 9600 data points on the x-axis. It seems that the jump in sensitivity occurs at a single value of change in R.I. But, in actuality the jump is at the next data point. The same nature of the curve can be seen in Figure 6.5.¹¹²

$$S = \frac{abs(\Delta\theta_{res})}{\Delta n_3}$$

$$= \frac{abs(\arcsin Re \left(\frac{1}{n_1} \sqrt{\frac{n_2^2 n_3^2}{n_2^2 + n_3^2}} \right) - \arcsin Re \left(\frac{1}{n_1} \sqrt{\frac{n_2^2 (n_3 + \Delta n_3)^2}{n_2^2 + (n_3 + \Delta n_3)^2}} \right))}{\Delta n_3} \quad (6.3.1).$$

Figure 6.2. Sensitivity variation with changes in RI of the analyte for the SPR Kretschmann configuration at various wavelengths of 690 nm, 780 nm and 830 nm.¹¹²

The respective values of Haemagglutination Units (HAU) concentrations at these minimum RI values for the three wavelengths are: 690 nm – 0.0669 HAU, 780 nm – 0.033 HAU and 830 nm – 0.0855 HAU. The maximum sensitivities at these wavelengths are: 690 nm – 196.07 deg/RIU, 780 nm – 500 deg/RIU and 830 nm – 147.05 deg/RIU and the average sensitivity values are: 690 nm – 114.88 ± 25.48 deg/RIU, 780 nm – 122.4 ± 38.68 and 830 nm – 95.8 ± 24.69 . The average sensitivity has been arrived by considering the sensitivity values at RI changes from 1×10^{-5} to 1.1×10^{-3} . 1 HAU corresponds to a concentration of virus that is capable of completely agglutinating the red blood cells (RBCs).⁷³

$$y = 915.6x - 10.285 \quad (6.3.2).$$

In the above equation, y is the change in R.I. and x is the concentration in HAU. Using equation 6.3.2, the corresponding RI change for 1 HAU is 0.000905. In case of our SPR layout, since the HAU concentrations at these wavelengths are much below 1 HAU, we can argue that the SPR based bio-sensor can safely detect the minimum toxicity level. As seen from figure 6.2, the sensitivity values are high at low RI changes. This aspect can be really beneficial since bio-molecular detection of nano and micro molecules like viruses and bacteria result in small RI changes. Thus, having a high sensitivity value at low RI change of analyte will enable easier detection of pathogens.

Figure 6.3 displays the variation between the normal part of the electric field and the distance from the metal-dielectric interface. The decaying nature of the field can be seen here as this is an evanescent field that is maximum at the surface and slowly decreases as the distance from the surface increases. The penetration depth of this field at the three wavelengths are: 690 nm – 150.62 nm, 780 nm – 170.96 and 830 nm – 183.71 nm. Bio-molecular interactions that occur within this penetration depth are able to cause RI changes of the analyte and thus these interactions can then be detected.

Figure 6.3. Variation of normal component of electric field with the distance from the metal-dielectric interface for the SPR Kretschmann arrangement at various wavelengths of 690 nm, 780 nm and 830 nm.¹¹²

Two-dimensional (2D) materials constitute a fresh category of materials that possess some remarkable physical, optical and chemical properties. Among these materials, graphene can offer several benefits in SPR-based sensing. Graphene can contribute to significant boost in the electric field at the substrate interface that can lead to higher sensitivity because of increased plasmon generation. Additionally, they have a large surface area of about $2630 \text{ m}^2\text{g}^{-1}$ and thus can have greater interaction with the analyte. They can selectively attach to aromatic compounds through pi-pi interactions. Hence, graphene can easily capture small bio-molecules with this specific pi-pi bonding that they can form with the flowing analyte. Non-specific binding can cause major issues as it can lead to erroneous sensorgram curves. The non-specific binding sites can be blocked by using Polyethylene Glycol (PEG) as a blocking agent. Apart from these features, graphene can now be commercially fabricated and is not just limited to research laboratories in universities. The coating of graphene over Gold is also a known procedure and can be performed at a mass scale in industry. Thus, we further analyze the graphene modified SPR configuration to have a detailed look into the enhancement in sensitivity and detection limits that it can give us.

For the Au + graphene layout, we first optimize N_{gh} . Using equations 4.1.1 through 4.1.7, we evaluate the sensitivity for this modified layout. Figure 6.4 shows the sensitivity change as a function of graphene layers. We can observe a general trend here. The sensitivity increases as the graphene layers increase up to a specific limit beyond which the sensitivity starts falling down. With added graphene layers, there is enhanced light-matter coupling that enables more surface plasmons to be produced and thus the sensitivity multiplies. However, when the number of graphene layers exceeds a certain value, the effect is counterproductive as it causes the incident photons to get absorbed because of which less photons are able to interact with the plasmonic metal causing reduced generation of plasmons. The number of layers of graphene that gives us the highest sensitivity is our optimized condition. The optimized condition for the three wavelengths are: 690 nm – 15 layers, 780 nm – 22 layers and 830 nm – 26 layers.

Figure 6.4. Variation of sensitivity as a function of number of graphene layers for the SPR structure modified with graphene at various wavelengths of 690 nm, 780 nm and 830 nm.¹¹²

In the modified Au + graphene layout, we vary the RI of analyte from 5×10^{-6} to 1.1×10^{-3} and observe the sensitivity. Figure 6.5 displays the variation of sensitivity with changes in the RI of the analyte for the graphene modified SPR layout. This is similar to Figure 6.2 except that 15, 22 and 26 layers of 0.34 nm graphene for the corresponding wavelengths of 690 nm, 780 nm and 830 nm have been added as additional layers between Au and the analyte. The minimum detection limits for the Au + graphene configuration in terms of change in RI are: 690 nm – 7×10^{-6} , 780 nm – 1.1×10^{-5} and 830 nm – 4×10^{-5} . Also, the respective values of HAU concentrations at these minimum RI values for the three wavelengths are: 690 nm – 0.0188 HAU, 780 nm – 0.023 HAU and 830 nm – 0.054 HAU, the maximum sensitivities at these wavelengths are: 690 nm – 1428.6 deg/RIU, 780 nm – 909.09 deg/RIU and 830 nm – 250 deg/RIU and the average sensitivity values are: 690 nm – 172.04 ± 86.04 , 780 nm – 156.93 ± 60.63 and 830 nm – 132.17 ± 27.75 . If we compare the Au + graphene layout with the standard Kretschmann configuration, the detection limits are lowered by 4.4×10^{-5} @ 690 nm, 9×10^{-6} @ 780 nm, 2.8×10^{-5} @ 830 nm and the average sensitivities are enhanced 1.49 times @ 690 nm, 1.28 times @ 780 nm, 1.37 times @ 830 nm.

For both only Au configuration and Au + graphene layout, at the maximum sensitivity data point, the change in SPR angle is 0.01 degree for all wavelengths. For commercial sensors, a 1000 RU change in the response corresponds to a shift of 0.1 degree. Thus, for our case, this corresponds to a change of 100 RU.⁷³

$$Y=119.18\ln(X)-53.188 \quad (6.3.3).$$

Equation 6.3.3 shows the logarithmic relation between concentration of virus in nM (X) and change in response in RU (Y). A 100 RU change correlates to a concentration of 3.615 nM. Table 6.2 lists the detection limits for SPR detection of several respiratory viruses. The detection limits are: Influenza A – 5 nM, Influenza B – 1 nM, PIV1 – 1 nM, PIV2 – 2.5 nM, PIV3 – 3.5 nM, RSV – 3 nM, ADV – 0.5 nM, SARS – 2 nM and H1N1 – 3 nM. Considering these limits, we can argue that 3.615 nM is a reasonable value obtained as the detection limit from the simulation.

Figure 6.5. Sensitivity variation with changes in RI of the analyte for Au + graphene layout at various wavelengths of 690 nm, 780 nm and 830 nm.¹¹²

6.4 Conclusion

Plasmon-based bio-sensors are rapidly evolving as a label-free and real-time bio-physical detection technique capable of giving accurate and reliable results in a quick span of time. The unanticipated breakdown of the contagious COVID-19 virus has claimed many lives and has jeopardized the entire world with China being the worst affected country. In this work, we basically demonstrated with the help of simulation that SPR based bio-sensors can serve as an important tool for detection of respiratory viruses. We performed the simulation at three different operating wavelengths of some commercially available SPR sensors. With actual data from previous real-time bio-molecular SPR experiments on detection of Avian Influenza Virus we performed simulation at 690 nm, 780 nm and 830 nm for Au and Au + graphene layout and arrived at some key sensitivity and detection limit values. The Au layout gives a minimum limit of detection of 2×10^{-5} and a maximum sensitivity of 500 deg/RIU at 780 nm. For the Au + graphene configuration, the detection limit and sensitivity values are 7×10^{-6} and 1428.6 deg/RIU respectively at 690 nm. Additionally, using the experimental work done by Suenaga et al.⁷⁹, we developed a relation between concentration level and change in response in RIU. We found out that the SPR scheme can detect 3.615 nM of virus concentration. Hence, in clinical terms we can argue that the SPR scheme can detect concentration in the nM range.

Chapter 7

SARS-CoV-2 enhanced plasmonic detection with Au nanorods

Abstract- The beautiful interplay between light and matter can give rise to many striking physical phenomena, surface plasmon resonance (SPR) being one of them. Plasmonic immunosensors monitor refractive index (R.I.) changes that occur as a result of specific ligand-analyte or antibody-antigen interactions taking place on the sensor surface. The COVID-19 pandemic has jeopardized the entire world and has resulted in economic slowdown of most countries. In this work, we present a model of a sandwich plasmonic bio-sensor that utilizes gold nanorods (Au NRs) for the detection of COVID-19 SARS-CoV-2 spike protein. We present simulation results for different prismatic configurations for the basic Kretschmann layout. We find that a BK7 glass prism-based SPR sensor has an incremental sensitivity of 111.11 deg/RIU. Additionally, using Comsol Multiphysics we discuss in depth the electric field enhancement observed for various aspect ratios (ARs) and layouts of Au NRs. Our aim here is to show how simulation can be used in the preliminary stages of design of Au NRs based SPR sensors to decide upon the parameters of aspect ratio, wavelength and distance from the sensor surface.

7.1 Introduction

A sudden outbreak of several unexplained instances of pneumonia in Wuhan, China in the last week of December 2019 alarmed the medical community. Later, three months down the line, the scenario changed completely and the local outbreak in China spread globally and paralyzed 200 countries which included United States of America and many European countries as the ones who were badly affected. The outbreak was found to be caused by a unique strain of coronavirus (CoV) that was named as the novel coronavirus disease (COVID-19). The World Health Organization has declared the situation to be a pandemic where it is still evolving in many countries like India. The COVID-19 virus has symptoms similar to earlier outbreaks caused by the Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) CoVs. Both COVID-19 and SARS-CoV viruses have the same structure with 70% match in the sequence of their constituent amino acids. Also, their mode of entry into the human body is by binding with the same receptor called angiotensin-converting enzyme 2 (ACE 2). The COVID-19 virus was thus named as SARS-CoV-2. All the three viruses (SARS-CoV, MERS-CoV and SARS-CoV-2) are positive stranded RNA viruses that belong to the betacoronaviride family. The virus can cause mild to acute respiratory infections and has varied symptoms like fever, cough, difficulty breathing, loss of taste and smell. Due to its ability to mutate into different forms, its detection has not been easy. Elderly people, infants, adolescent children and people with compromised immune system are the ones at maximum risk of contracting the virus.

The SARS-CoV-2 genome comprises of 27 proteins that includes four structural and other non-structural proteins. The four structural proteins are the spike (S), envelope (E), nucleocapsid (N) and matrix (M) proteins. Being a surface protein, the S protein is primarily responsible for entry into the host cell. The virus was initially diagnosed using molecular techniques like reverse transcription polymerase chain reaction (RT-PCR). However, considering the increasing number of cases, the PCR kits have not been able to meet the testing demand. Also, underdeveloped and developing countries where there are many people who still live in rural areas, lack proper PCR infrastructure in rural and suburban areas. Lastly, since the technique detects the genome structure of the live virus present in the body, it cannot identify recovered asymptomatic patients. Thus, there has been ongoing research on finding easier and reliable detection methods. Serological testing has long been used for the detection of many viruses like Human Immunodeficiency Virus (HIV), SARS-CoV and MERS-CoV. This testing scheme mainly utilizes the reactivity between specific antibodies developed by the human immune system in response to the presence of viral antigens.

Localised surface plasmon resonance (LSPR) is a unique phenomenon observed in noble metal

nanoparticles (MNPs). When the incident EM light wave falls on the MNPs, the electric field interacts with the free electrons and causes them separate from the metal core. The repulsive force acting amongst the electrons cause them to move in opposite directions that results them to oscillate and thus excite the LSPR. The excitation of LSPR can induce strong light absorption. Au NRs have been enormously studied for many decades and have been used in varied applications like plasmonic photothermal therapy and cancer imaging, and even enhanced sensing and detection. Au NRs generally have two absorption bands namely longitudinal plasmon band (LPB) and transverse plasmon band (TPB) where the electrons oscillate along the long and short axes of the NR respectively. The aspect ratio (AR) has almost no impact on the TPB whereas the increase of AR red-shifts the LPB. Additionally, the LPB is also highly sensitive to R.I. changes. Experimental studies also reveal the linear relationship between the AR and the peak wavelength of LPB. In our current work, with the help of simulation, we demonstrate how Au NR can be utilized for signal amplification of conventional SPR sensors. Thus, using the antigen-antibody interaction principle, we describe a sandwich-type plasmonic immunoassay model that uses Au NR for enhancement of output signal.

Although we did not perform any experiments, Au NRs have been used in the past for successful amplification of optical response. Kim et al. used Au NR enhanced SPR scheme for the detection of norovirus capsid protein and were able to detect concentrations as low as 70 aM that was 105 times better than the normal SPR scheme without NR.¹¹³ Sim et al. observed 108 times signal enhancement compared to conventional SPR measurements by using biofunctionalized Au NRs for the detection of Immunoglobulin E (IgE).¹¹⁴ Lin et al. used a Au NR based plasmonic sensor to detect orchid viruses and found that the detection limits were 25 times lower than the conventional enzyme-linked immunosorbent assay (ELISA).¹¹⁵ Zhang et al. and Wang et al. have also used Au NRs for plasmonic detection of bovine IgG and hepatitis B virus.¹¹⁶⁻¹¹⁷

7.2. Sensor Structure

The conventional SPR scheme consists of a plasmonic metal, mainly gold, coated on a high R.I. prism. The structure of our Au NR modified layout is shown in Figure 7.1. In this sandwich-type structure, the gold nanosheet is functionalized with SARS-CoV-2 spike (S) protein antibody (Ab). Additionally, Au NRs are conjugated with the same Ab. The antigen is the SARS-CoV-2 virus in serum/nasopharyngeal swab samples. The procedure of immobilization starts with functionalization of the Au nanosheet with the S protein Ab. The serum samples that contain the antigen is allowed to flow as an analyte. And lastly, the Ab conjugated Au NR is applied for sensitivity enhancement. The electric field enhancement and consequently the improvement in sensitivity depends on many factors like the Au NR AR, the placement of the NR or the distance between the nanosheet and the NR and also the working wavelength of the sensor. Choosing a proper Au NR that has the peak absorbance near the working wavelength can ensure maximum coupling of local electric fields of Au NR and the evanescent field at the sensor surface. In section 7.4, we discuss in depth about how the placement of Au NR can affect the field enhancement and thus it constitutes an important parameter that needs to be assessed while designing immunosensors.

7.3 Simulation Technique

Most commercial SPR sensors work in the NIR region of light wave. Spreeta SPR sensors manufactured by Texas Instruments are miniaturized SPR sensors that are widely used for research purposes. A wide variety of commercial SPR sensors are available in the market like Biacore manufactured by GE, SPRm 200 manufactured by Biosensing Instrument, Carterra LSA SPR platform, IBIS-MX96 by IBIS Technologies, SensiQ pioneer by SensiQ technologies. However, all these sensors are bulky and are not designed to meet the needs of point-of-care (POC) diagnostic technique. Spreeta SPR sensors are low-cost portable sensors that can be widely deployed for designing POC SPR detection kits. The sensor can provide detection results within a few minutes compared to other laborious methods like Enzyme-linked Immunosorbent Assay (ELISA) that can take several hours. Hence, we perform the simulation at the working wavelength of these sensors $\lambda = 830$ nm. The working R.I. range of these miniature sensors are from 1.32 to 1.368 with a precision of 5×10^{-6} RIU. Thus, we vary the R.I. of the analyte layer from 5×10^{-6} to 4.8×10^{-2} in steps of 5×10^{-6} and calculate the change in SPR angle $\Delta\theta_{SPR}$. The thickness of the gold nanosheet is 50 nm and we carry out the simulation for three kinds of prism namely SF10, SF11 and BK7. The R.I. of the three prisms at 830 nm are 1.7099, 1.7630 and 1.5102 respectively. Additionally, the R.I. of Au at the working wavelength is $0.2328 + i4.8144$. The first layer of the sensor is the high R.I. prism followed by the second layer of Au, and the third layer is the analyte which is considered as de-ionized (D.I.) water whose R.I. is chosen to be 1.33.

Figure 7.1. Structure of the plasmonic immunoassay based on Au NR and sandwich-type assembly using antigen, primary and secondary antibodies.¹¹⁸

Since SPPs are electron density waves, the evanescent electric field simulation can provide us a good insight into the sensitivity. The normal component of the electric field decays exponentially as one moves away from the sensing surface towards the analyte. The penetration depth is defined as the distance at which the field becomes $1/e$ of its value at the sensing surface. Bio-molecular interactions that take place in this depth can result in RI changes of the medium. Increased electric field can enhance the penetration depth and thus the probability of the interaction getting detected increases. Hence, using Finite-difference Time Domain (FDTD) simulations which provide data about the magnitude and penetration depth into the analyte for the electric field of the SPPs at the Au film sensor surface and LSPR waves oscillating along the longitudinal axis of the Au NR. For the electric field simulation, we use Comsol Multiphysics. The diameter of the Au NR is chosen to be 10 nm. We vary the aspect ratio (AR) from 1 to 4 and the distance of the Au NR from the Au nanosheet from 2 nm to 14 nm in steps of 3 nm. Therefore, in totality we evaluate 20 different scenarios by taking all the combinations of ARs and Au NR distance from nanosheet.

7.4 Results and Discussion

We perform the simulation at 830 nm. The SPR reflectivity, which is the ratio between incident and reflected light intensities, has been shown in Figure 7.2 for different scenarios. After crossing the critical angle of incidence, the reflected light undergoes Total Internal Reflection (TIR). At a particular incident angle known as the SPR angle, the reflectivity is minimum. Beyond this angle, the reflectivity again starts to increase. Figure 7.2a shows the SPR reflectivity curve for Au NR with an AR of 1 whereas Figure 7.2b shows the same for AR=4. “s” is the distance of the Au NR from the Au nanosheet. The dip in the reflectivity is due to plasmonic resonance and at the resonance occurs at the minimum reflectivity point. For the angular interrogation technique, we evaluate three different prismatic configurations. By varying the R.I. of the analyte from 5×10^{-6} to 4.8×10^{-2} in steps of 5×10^{-6} , we observe the corresponding change in SPR angle. Additionally, for most commercial sensors, the change in R.I. that occurs due to ligand-analyte interaction is represented in the sensorgram, a real-time response curve, in the form of change in sensor response. A change in 0.1 degree in the SPR angle results in a change of 1000 RU in the sensorgram response. Thus, Figure 7.3 shows the respective SPR angle and sensorgram response changes for the three prism types namely SF10, SF11 and BK7. From the simulation results, we observe that the SPR angle changes in the form of steps. This is not very evident from the curve which shows a linear variation since

Figure 7.2. SPR reflectivity curve for the Au NR based SPR sensor with AR=1 and AR= 4 and different values of parameters, the distance between Au NR and Au nanosheet.¹¹⁸

the step size is very small. However, the figure inset shows the magnified step variation. The corresponding step sizes for SF10, SF11 and BK7 prism-based sensors are 1.5×10^{-4} , 1.65×10^{-4} and 9×10^{-5} RIU respectively. This means that only when the R.I. changes by these amounts, the respective sensors can detect a change in the SPR angle. Hence, the lower the step size, the better the sensor resolution. Hence, BK7 prism-based sensor gives the best sensor resolution among the three prism types. The limit of detection (LOD), which is the minimum R.I. change the sensor can detect, for the SF10, SF11 and BK7-prism based sensors are 4×10^{-5} , 8.5×10^{-5} and 7×10^{-5} RIU respectively. Thus, in terms of LOD, SF10 prism-based sensor gives the best result. If we talk about incremental sensitivity, defined as the ratio of change in SPR angle to the step-size change in RI, the values for the SF10, SF11 and BK7 prism-based sensors are 66.67, 60.60 and 111.11 deg/RIU respectively. Hence, in terms of incremental sensitivity, BK7 prism-based sensor gives the ideal performance. Thus, designing sensors based on BK7 prism can detect small RI changes easily and hence help in detecting lower concentrations of SARS-CoV-2 S protein antigen.

Figure 7.3. Variation of SPR angle and sensorgram response as a function of change in R.I. of the analyte for (a) SF10 (b) SF11 and (c) BK7 prisms.¹¹⁸

Figure 7.4. Evanescent decay of the electric field penetrating into the analyte solution from the surface of Au nanosheet along the central line of Au NR at the resonance condition for (a) AR=1 and (b) AR=4 and different values of parameter s, the distance between Au NR and Au nanosheet.¹¹⁸

For simulation of electric field, we used Comsol Multiphysics. Briefly, the Au NR is represented as a two-dimensional (2D) rectangular structure with a width of 10 nm and varying heights depending on the AR. The simulation is carried out at 830 nm. We consider the effect of different ARs from AR=1 to 4 and varying distances of 2,5,8,11 and 14 nm between the NR and the Au nanosheet. Figure 7.4 below shows the evanescent field variation as a function of distance from the plasmonic nanosheet. As can be seen the field gradually decreases as one moves away from the metal nanosheet and hence the name evanescent. The penetration depth of this field is defined as the distance at which the field is $1/e$ times its value at the metal surface. The field normally vanishes and becomes negligible at one-fourth to one-third wavelength distance. All ligand-analyte interactions that take place in between this zone can cause R.I. changes of the analyte. Hence, it is essential to keep the entire sensing system compact with minimum distance between the different layers. As can be observed from the evanescent curves here, there are local peaks in the electric field values. These correspond to local hot spots at the tips of the Au NR due to the excitation of plasmons at these areas giving rise to localized resonance or also known as LSPR. These sudden peaks in the field values can contribute to enhanced sensitivity especially when there is a matching of the LSPR wavelength of the Au NR and the SPR wavelength of the metal film underneath.

For the simple Au nanosheet configuration, the electric field is 2.45×10^5 V/m. The phenomenon of the plasmonic effect in the nanosheet and the NR are different in nature. While Au NR experiences LSPR, it is the SPPs that travel along the plasmonic metal nanosheet. The LSPR field is concentrated around the nanostructure and decays within 10-30 nm and is thus quite sensitive to distance from the metal sheet and the RI changes of the surrounding area. In contrast to this, SPPs are evanescent in nature that progressively reduce to zero within 100-200 nm distance. The confined electron cloud oscillation in the Au NRs can produce intense local EM fields that are several orders higher than the incident field. Upon the introduction of Au NR into the plasmonic nanosheet-based system, the SPPs travelling along the Au nanosheet can couple with the local EM oscillations at the Au NR tips that can lead to resonance and boost the decaying evanescent field by several orders of magnitude. Hence, the evanescent field perturbation caused by the NRs can lead to signal augmentation and corresponding enhancement in the sensitivity. Thus, incorporating Au NR can allow the SPPs to couple with LSPR EM field, leading to enhanced electric field and lower detection limits. We observe from Figure 7.4a and 7.4b that as we increase the distance between the plasmonic metal nanosheet and the Au NR, the enhancement in local field reduces. This can be attributed to reduced evanescent field coupling between the Au nanosheet and the Au NR. Thus, the field enhancement is highest for a distance of 2 nm between the sheet and the NR and lowest for 14 nm.

Figure 7.5 shows the electric field distribution across the sensor surface at the resonance condition. The local field enhancement at the tip of the Au NRs can be easily seen as red-hot spots. The distribution follows the same trend as in Figure 7.4. The field reduces as one moves away from the nanosheet but peaks at the tips of the Au NRs before finally decaying down. The electric field and the corresponding enhancements for all the twenty combinations (4 different ARs X 5 different distances between the sheet and the NR) of Au NR and Au nanosheet arrangement have been summarized in Table 7.1. We observe

Figure 7.5. Normal electric field distributions at the resonance condition for Au NR with different aspect ratios and located at various distances s from the Au nanosheet for AR=1 (a-e) and AR=4 (f-j).¹¹⁸

that as we increase the AR, the local field enhancement increases. For our sandwich-type assay, the immobilization with S protein Ab and the flow of antigen containing serum samples can take up about 10-15 nm distance on top of the sensing film. With compact packing of the different layers, this can be reduced to 10 nm. Thus, to counteract the reduced enhancement of the local electric field due to increased distance between the Au nanosheet and the Au NR, we need to choose higher aspect ratios. Hence, for our case, we observe that by choosing a Au NR with AR=4 that is located 14 nm above the Au nanosheet, we can get an enhancement of 376.33%.

The AuNR-based SPR model present here can be effectively realized and designed using commercial Spreeta SPR sensors. With features like real-time sensing, quantitative analysis, internal fault detection mechanism, robust design, small and lightweight, these sensors can be efficiently used for diagnostics. With a volumetric dimension of 1.63 cm X 1.139 cm X 0.531 cm, and an in-built integrated circuit (IC) chip system, they are quite compatible for the design of POC devices where they can be merged together with other software and hardware platforms to design a complete wireless plasmonic sensing scheme that encompasses an electronic wireless transmission module that can store the test results in the data cloud for global data monitoring and analysis. The reason behind choosing gold nanorods is due to its easy and comparatively simple method of preparation as compared to other nanomaterials. Additionally, commercially prepared Au NRs with different aspect ratios having a particular LSPR wavelength can be procured easily as the synthesis method is quite mature and many chemical companies can actually prepare customized Au NRs. Also, there have been established methods of synthesizing customized Au NRs. The seed-mediated growth method forms the basis of many improved methods. By tweaking various parameters, many researchers have come up with different techniques of preparing fine-tuned Au NRs where they can control the AR by varying the reaction conditions like temperature or the concentration of a particular solvent. Given the high bio-compatibility of these Au NRs, they can be conjugated to Abs in a facile and efficient way. For instance, they can attach themselves to the Abs by various mechanisms like Abs being electrostatically adsorbed on the Cetyl Trimethylammonium Bromide (CTAB) layer on the Au NRs; coating the NR CTAB layer with charged polymers and following it with electrostatic adsorption of Abs; replacing the CTAB layer with biofunctional ligand to which the Abs can attach through covalent binding; and replacing the CTAB layer with methoxypoly(ethyleneglycol)(mPEG)-thiol molecules and following it with directional conjugation that takes place with the help of the carbohydrate portion situated on the heavy chains of the Fc region of antibodies. The chemical binding of the various constituents can affect the R.I. of the analyte. In a qualitative way, the R.I. change affects the change in SPR angle and thus the change in sensorgram response. Thus, due to experimental errors if the binding is not ideal and results in a decreased R.I. change, the sensorgram response will be reduced. The virus can still be detected but the concentration deduced from the response will be lower than the actual. Considering all these aspects, we believe that the actual model of the proposed plasmonic immunosensor can be fruitfully realized. We would also like to emphasize that the use of Au NRs can not only help this particular

Table 7.1. Enhancement of electric field for various arrangements of Au NR as compared to the standard Au nanosheet (without Au NR)

Distance from nanosheet	2 nm	Enhancement (%)	5 nm	Enhancement (%)	8 nm	Enhancement (%)	11 nm	Enhancement (%)	14 nm	Enhancement (%)
Au nanosheet (without NR)	2.45×10^5	-	2.45×10^5	-	2.45×10^5	-	2.45×10^5	-	2.45×10^5	-
Au nanosheet + Au NR with AR=1	7.42×10^5	302.86	4.91×10^5	200.41	4.25×10^5	173.47	4.39×10^5	179.18	3.95×10^5	161.22
Au nanosheet + Au NR with AR=2	1.28×10^6	522.45	7.68×10^5	313.47	6.51×10^5	265.71	6.16×10^5	251.43	5.39×10^5	220
Au nanosheet + Au NR with AR=3	1.86×10^6	759.18	1.03×10^6	420.41	8.96×10^5	365.71	7.98×10^5	325.71	7.23×10^5	295.10
Au nanosheet + Au NR with AR=4	2.5×10^6	1020.41	1.34×10^6	546.94	1.17×10^6	477.55	1.02×10^6	416.33	9.22×10^5	376.33

miniaturized version of the sensing system but can also immensely improve the sensitivity of commercial SPR systems.

7.5 Conclusion

The rapidly rising cases of COVID-19 has placed enormous stress on the medical fraternity. The easy transmissibility of the COVID-19 virus has led to an exponential increase in the number of infected people. Thus, faster and efficient diagnostic techniques are required that can detect even trace amounts of the SARS-CoV-2 virus. Plasmonic sensing scheme offers many advantages like label-free and real-time detection technique. Thus, in this work we present a sandwich-type plasmonic model that makes use of Au NRs to enhance the sensitivity by virtue of magnification of electric field. We present simulation results for various ARs and arrangements of the Au NRs. We find that increasing the AR can effectively lead to improved electric field. Alternatively, increasing the distance between the Au NR and the plasmonic Au nanosheet can reduce the evanescent field. For a typical 10 nm diameter Au NR-based configuration (Au nanosheet + Au NR with AR=4), we observe an evanescent field improvement of 376.33% as compared to the basic Au nanosheet-based Kretschmann model.

Chapter 8

Comparison of plasmonic performance of 2D materials

Abstract— Plasmonic sensing relies on the interaction between the electromagnetic light wave and free electrons that reside at the interface of two materials, one of them being a metal. Surface Plasmon Resonance (SPR)-based bio-sensing has distinct advantages of providing real-time and label-free detection technique that is capable of giving accurate and repeatable results. Two-dimensional (2D) nanomaterials (Graphene and Transition Metal Dichalcogenides (TMDCs) like MoS₂ and WS₂) have recently gained high popularity in the field of biomedical sciences. Using Biacore commercial SPR machine manufactured by GE Healthcare, we evaluate the efficacy of various combinations of these materials coated on the plasmonic metal Gold (Au) (Au+MoS₂, Au+WS₂, Au+graphene, Au+graphene+MoS₂ and Au+graphene+WS₂), in enhancing the sensitivity of the biosensor as compared to the standard Kretschmann configuration (only Au). We also present some simulation results at incident light wavelengths of 633 nm and 785 nm that are based on the angular interrogation method that uses the Transfer Matrix Method (TMM) and Fresnel's Reflection Laws for the calculation of angular sensitivity of the SPR sensor. To conclude, we find that the sensitivity in the increasing order is: only Au < Au+MoS₂ < Au+WS₂ $\approx$ Au+graphene < Au+graphene+MoS₂ < Au+graphene+WS₂. The main aim of carrying out this experiment is to find the best sensor layout that can be further used for protein-protein bio-sensing interaction and Goos-Hanchen (GH) shift optical experiments.

8.1 Introduction

Biacore systems utilize SPR technology to study bio-molecular interactions in real-time. This involves attaching an interacting molecule, usually called a ligand, to the sensor chip and passing sample solutions that contain another molecule, which can bind to the ligand immobilized on the sensor surface. The Biacore system can study specificity, kinetics and affinity of biomolecular interactions and can also measure the concentration of specific molecules. The primary components of the system are the sensor chip, the optical setup and the microfluidic system. The sensor chip comprises of thin-film gold (about 50 nm) coated on a glass slide. The glass slide of the sensor chip is pressed against a semi-cylindrical glass prism using a silicone opto-interface. A near-infra red (NIR) light emitting diode (LED) acts as the optical excitation source. The microfluidic system has a series of flow channels and valves enclosed in a plastic block, the Integrated Microfluidic Cartridge (IFC) that supplies a continuous flow of running buffer and sample solution in a controlled manner with the help of a delivery pump. The pump ensures proper switching intervals between the buffer and the sample solution ensuring minimum disruption and diffusion on the sensor surface. The response of the system is measured in resonance units (RU) and it is directly proportional to the concentration of biomolecules on the surface. The sensorgram displays an interactive real-time response curve that is indicative of the various processes taking place on the sensor surface. One of the advantages of the SPR technology is in the reusability of the sensor chips. The revival process is usually done at the end of an analysis cycle where a regeneration buffer helps in the removal of bound analyte from the sensor surface causing minimal structural deformation of the ligand molecule.

2D materials (Graphene and TMDCs) are a new class of nanomaterials that have atomically thin dimensions of the order of 1 nm. The TMDC family has more than forty compounds and they are in generic form denoted by MX₂, where M is a transition metal from groups IV to VII of the periodic table and X is a chalcogen like S, Se and Te. In its structural form, the transition metal is sandwiched between the two chalcogen atoms to form a three-layered stack held together by weak Van-der Waal's forces of attraction. The scaling down of the bulk TMDC to a single layer completely transforms its optical properties. The indirect bandgap of the bulk material is metamorphosed to a low direct bandgap of the order of 1.8 eV that is comparable to the bandgap of semiconducting materials like Silicon (Si). This transformation to a low bandgap nanomaterial facilitates easy absorption of photons that can directly excite the electrons from the valence band to the conduction band, without the need of additional phonons, which are required in case of indirect bandgap materials. Thus, because of unique physical, mechanical

and optical properties of these 2D materials, their application areas are being actively researched upon like in their use in high-index dielectric Mie nanoresonators, photodetectors, optical modulators [24], infra-red plasmonic devices. These 2D materials have intrinsic plasmons that can be tuned by gating and doping. Combining graphene and other TMDC materials with plasmonic nanostructures can enhance light-matter interactions. And this structural enhancement has been employed in many optoelectronic devices. For instance, graphene photodetectors that are modified by plasmonic structures have 15-20 times amplification in the photovoltage. Moreover, because of high surface-to-volume ratio of the 2D materials, they are very sensitive to changes in their surroundings. There can be variations in charge transfer and doping, intercalation and also prominent alternations in their permittivity and lattice vibrations. And this makes them apt for molecular sensing. Additionally, these materials have also enormously contributed to advancements in the field of cancer theranostics, preventive medicine, drug-delivery and bio-sensing.

8.2 Results and Discussion

The experimental procedure using the Biacore T200 SPR machine comprises of establishing baseline stability using HBS (HEPES buffered saline) buffer, a zwitterionic sulfonic acid buffering agent. At fixed time intervals, different concentrations (25%, 50% and 100%) of standard BIAtest solution (15% (w/w) sucrose in HBS-N buffer) are injected at a fixed flow rate and the response is observed in the sensorgram. Figure 8.1 shows the response (average of response from four flow channels of the IFC) measured in RU (resonance units) as a function of time for the six different sensor configurations. The injection of sample solution (sucrose in HBS-N buffer) causes a sharp sudden rise in response due to a change in refractive index (the R.I. of pure HBS buffer changes when the sample solution is injected). Since there is no binding of molecules to the sensor surface, the response returns to the original signal level after 30 seconds of sample flow. Increased concentration results in greater R.I. changes and hence the response is enhanced for higher concentrations.

Table 8.1 below summarizes these calculations. In terms of the enhancement levels in percentage points, we can conclude that the sensitivity order is: only Au < Au+MoS₂ < Au+WS₂ $\approx$ Au+graphene < Au+graphene+MoS₂ < Au+graphene+WS₂. Thus, heterolayer 2D structures of graphene and TMDC offer better results than monolayer 2D structures. This can be attributed to enhanced light-matter coupling in heterolayer structures because of the presence of an additional layer of 2D material.

8.3 Simulation of the SPR structure

8.3.1 Angular Sensitivity Calculation

Using Fresnel's Reflection Laws and the Transfer Matrix Method, we evaluate the angular sensitivity of six different configurations (only Au, Au+MoS₂, Au+WS₂, Au+graphene, Au+graphene+MoS₂ and Au+graphene+WS₂) at two different wavelengths of 633 nm and 785 nm respectively using equations 4.1.1 to 4.1.7.

For the six different structures studied here, N=3 for the standard Kretschmann configuration (only Au), N=4 for Au+MoS₂, Au+WS₂ and Au+graphene and N=5 for Au+graphene+MoS₂ and Au+graphene+WS₂. For all these configurations, the first layer comprises of the SF10 glass prism and the last layer is the analyte medium that is taken to be deionized (D.I.) water. The intermediate layers are made up of various combinations of the plasmonic metal Au and the 2D materials (MoS₂, WS₂ and graphene). For Au, the thickness considered is 50 nm and for every 2D material, we consider its thickness to be 1 nm.

Figure 8.2 shows the basic SPR reflectivity curve at 785 nm. The dip in the reflectivity is due to plasmonic resonances. Many literatures show the coupling of plasmonic resonances in Au with excitonic resonances in semiconductor-based materials especially TMDC materials like WS₂ and MoS₂. However, the strong plasmonic-excitonic coupling needs certain conditions to be met. The light-matter interaction in excitonic-plasmonic coupling is generally explained in a microcavity environment. The strong coupling is defined by three parameters: g = energy transfer rate between light and matter, κ = rate of light escape from the cavity and γ = rate of matter loses its polarization. For the coupling to take place, the first energy term

Fig. 8.1. SPR Sensor response obtained using Biacore T200 for different kinds of arrangements (basic Au and Au modified with TMDC materials) tested using standard BIATest sucrose solution.¹¹⁹

should be greater than the sum of the other two energy terms or $g > \kappa + \gamma$. As seen in many literatures, the plasmonic excitonic coupling demonstrated by the researchers were specifically designed by special spatial arrangements of the plasmonic metal and the TMDC material that leads to the formation of a suitable microcavity environment. For instance, the use of Au nanorods or Au nanoarrays in the form of pillars or caps. In our SPR configuration, we have a layered configuration ($\text{MoS}_2/\text{WS}_2/\text{graphene}$) on top of Au film and thus do not have a suitable microcavity environment that can help in the coupling of excitons and plasmons. Hence, a resonance splitting which is a characteristic feature of the tuning of plasmonic and excitonic modes of resonances cannot be observed here.¹²⁰⁻¹²³ Table 8.2 shows the summary of simulation results at 785 nm. We observe that there is an enhancement in sensitivity upon addition of 2D materials as intermediate layers. This can be explained by the fact that additional layers enhance the light matter coupling that accentuates the evanescent field. Since the penetration depth of this field is defined as the distance at which the field is $1/e$ of its maximum value, a higher value of the evanescent field at the plasmonic metal surface ensures greater travelling depth. Since bio-molecular interactions can be detected within the penetration depth of this field, a higher depth ensures greater chances of detection. We can also see that the heterostructure configurations ($\text{Au}+\text{graphene}+\text{MoS}_2$ and $\text{Au}+\text{graphene}+\text{WS}_2$) can further enhance the sensitivity. Thus, the trend of the simulation results is quite similar to that obtained in the experimental results using the commercial sensor. Thus, heterolayer structures of combinations of graphene and TMDCs offer better sensitivity results than monolayer structures made out of either the TMDC material or graphene.

Table 8.1 Summary of enhancement values for different sensor configurations

S. No.	Sensor configuration	Average of % enhancements for changes in mean responses as compared to Au substrate	% Enhancement of average change in mean response as compared to Au substrate
1.	Au+MoS ₂	2.19	1.69
2.	Au+WS ₂	2.26	1.77
3.	Au+graphene	3.61	1.70
4.	Au+graphene+MoS ₂	3.25	2.46
5.	Au+graphene+WS ₂	4.66	3.13

Fig 8.2 SPR reflectivity curve at 785 nm for various SPR structures (Au and Au modified with TMDC layers).¹¹⁹

Table 8.2 Summary of simulation results at 785 nm for $\Delta n_{bio} = 0.005$.

Scenario	Minimum Reflectivity (R_{min}) (in a.u.)	SPR Angle 1 (θ_{SPR1}) (in degree)	SPR Angle 2 (θ_{SPR2}) (in degree)	Sensitivity $\left(\frac{\theta_{SPR2}-\theta_{SPR1}}{\Delta n_{bio}}\right)$ (in degree/R.I.U)	Sensitivity enhancement (in %)
Only Au	1.03E-2	54.48	54.81	66	---
Au+graphene	7.08E-2	54.80	55.14	68	3.03
Au+WS ₂	1.13E-2	54.94	55.28	68	3.03
Au+graphene+WS ₂	7.25E-2	55.30	55.65	70	6.06
Au+MoS ₂	1.43E-2	55.04	55.38	68	3.03
Au+graphene+MoS ₂	7.76E-2	55.41	55.76	70	6.06

Fig 8.3 Evanescent field distribution for various sensor layouts at 785 nm for different arrangements using basic Au and Au coated with TMDC materials.¹¹⁹

8.3.2 Electric Field Distribution

SPs propagate along the plasmonic metal surface. The SPW has a decaying perpendicular component also known as the evanescent field. This component travels along a direction normal to the metal-dielectric interface. Using Comsol Multiphysics, we evaluate the normal component of the electric field at the excitation wavelength of 785 nm. For the purpose of simulation, we take the depth of the analyte region as 1000 nm. Figure 8.3 shows the variation of the evanescent field as a function of distance from the metal surface as it traverses towards the analyte region. As observed, the evanescent field can be increased by incorporating intermediate layers of 2D materials. Table 8.3 shows the details of the electric field values. It gives the maximum value of this field observed at the sensor surface. MoS₂, WS₂ and other TMDC materials possess unique qualities of small direct band gap, high optical absorption in the visible and near infrared region, high surface-area to volume ratio. All such properties enable greater light-matter

Table 8.3 Summary of the evanescent field values for different sensor layouts at 785 nm

Scenario	Magnitude of normal component of electric field at the surface (norm. E) (in V/m)	Enhancement in evanescent field (in %)	Penetration depth of the evanescent field (in nm)	Enhancement in penetration depth (in %)
Au	3239.81	---	111.88	---
Au+MoS ₂	3430.52	5.89	114.99	2.78
Au+WS ₂	3375.62	4.19	114.98	2.77
Au+graphene	3276.38	1.13	114.99	2.78
Au+graphene+MoS ₂	3555.07	9.73	115.42	3.16
Au+graphene+WS ₂	3495.35	7.89	115.42	3.16

interaction that helps in the generation of more surface plasmons. The heterolayer structures can provide maximum enhancement. The Au+graphene+MoS₂ and Au+graphene+WS₂ configurations boost the electric field at the surface by 9.73% and 7.89% respectively as compared to the standard Kretschmann configuration (only Au). The penetration depth of the evanescent field, L_p , is defined as the distance at which the field becomes 1/e of its value at the plasmonic metal surface. The penetration depth of the field is improved by 2.77% and 3.16% for the monolayer modified configurations (Au+MoS₂, Au+WS₂ and Au+graphene) and heterolayer modified configurations (Au+graphene+MoS₂ and Au+graphene+WS₂) respectively as compared to the standard Au only structure.

8.4 Conclusion

Surface plasmon polaritons (SPPs) are electromagnetic waves that are generated by light-matter interactions. The physical phenomenon of SPR, which was discovered more than two decades back, has been a boon to the bio-medical industry. With several benefits like repeatability of results, reusability of sensor chips and efficient detection process, SPR-based bio-sensors have been able to make a mark in the field of bio-medical sensing. The research on fusion of nanotechnology with life sciences has been an ongoing process. 2D materials like graphene and TMDCs have unique physical and optical properties like high surface area, weak Vander Waal's interaction and high optical absorption. In this article, we take a look at the ability of these nano-materials in enhancing the sensitivity of SPR sensors. In brief, with the help of a commercial SPR sensor, we compare the efficacy of five different modified SPR configurations (Au+MoS₂, Au+WS₂, Au+graphene, Au+graphene+MoS₂ and Au+graphene+WS₂) with that of the basic Kretschmann arrangement. Summarizing the outcome, the experimental results indicate the sensitivity order to be; only Au < Au+MoS₂ < Au+WS₂ $\approx$ Au+graphene < Au+graphene+MoS₂ < Au+graphene+WS₂.

Chapter 9

Protein-protein interaction study

Abstract- The interplay between light photons and nanomaterials can give rise to many striking phenomena. Surface Plasmon Resonance (SPR), which is one such plasmonic sensing mechanism is gaining huge attention from the bio-medical fraternity. Two-dimensional (2D) nanomaterials like Transition Metal Dichalcogenides (TMDCs) and graphene are recently discovered nanomaterials that have favorable properties like high optical absorption. These materials have been applied extensively in the field of drug delivery and cancer therapy. However, its application in the area of plasmonic bio-sensing needs to be explored more. Hence, using commercial SPR sensors, we demonstrate the effectiveness of hetero-structured monolayer nanosheets of graphene and WS₂ for a plasmonic-based detection system of a specific ligand-analyte interaction between Bovine Serum Albumin (BSA) and Anti-BSA. Our detection technique does not require any complex functionalization scheme as the graphene-WS₂ nanosheet coated gold chip can attach protein molecules through hydrophobic interactions. The limit of detection (LOD) obtained for the Anti-BSA protein is 0.44 µg/ml. Thus, similar to earlier plasmonic experiments carried out using graphene and MoS₂ by incorporating a custom-made setup, we demonstrated that TMDC based sensing using Graphene-WS₂ can also be extended to commercial SPR sensors for a hassle-free system that does not require complex functionalization protocols.

9.1 Introduction

2D structures like graphene and WS₂ are newly discovered nanomaterials that have excellent features like high optical absorption and good mechanical robustness. Owing to their high electrical conductivity, these materials have many applications in optoelectronics in the form of sensors and photodetectors. They have robust in-plane covalent bonding and fragile Van der Waal's interlayer forces, due to which they can be easily exfoliated and drawn out into a single layer or several monolayers. This specific property also makes these materials highly flexible and thus they have been used in making flexible substrates for many sensing applications. Apart from the area of electronics, 2D materials have another altogether different arena of applications in bio-medicine and bio-sensing. Graphene has been phenomenally used as nano-devices and nano-agents for cancer diagnosis and therapy. Many TMDC materials like MoS₂ and WS₂ have been used in Deoxyribonucleic Acid (DNA) sensors, as photothermal agents and even as bio-imaging probes like photoacoustic imaging. Controlled light-assisted and pH-triggered drug release methods are nowadays being utilized for cancer therapy. These 2D nanomaterials have been used as nano-carriers for the delivery of many anti-cancer drugs like Doxorubicin (DOX) and Curcumin. Though these nanomaterials have been exquisitely researched for disease diagnosis and therapy, there are many other unexplored areas like plasmonic optical sensing. By employing these 2D materials as intermediate layers between Au and the analyte layer, the light-matter coupling can be significantly enhanced. This consequently leads to the generation of more plasmons resulting in enhanced sensitivity. Thus, in this present work, we use nano-sized monolayers of graphene and WS₂ for enhanced sensing of bio-molecular interactions. In this work, we make use of a commercial SPR machine to examine the sensing capabilities of 2D materials graphene and WS₂.

Identification of biomolecules using the SPR sensing platform requires structural modification of the plasmonic metal, which is usually done using chemical linkers like ((3-Aminopropyl) triethoxysilane, N-succinimidyl 4-Maleimidobutyrate and others. Many of these improvisations are complex, requiring a lot of effort, and additionally, they do not always give guaranteed outcomes. In this regard, the recently discovered TMDC materials, like MoS₂ and WS₂, can provide a promising insight. Certain favorable features like their hydrophobicity, enhanced surface area, and the presence of free Sulphur atoms, altogether make them a desirable candidate for the development of bio-sensing interfaces. The usage of MoS₂ nano-sheets provides a direct and facile method of immobilizing protein molecules on the sensor surface with the help of hydrophobic interactions. Though there have been quite a few earlier works on the plasmon-based detection of BSA, the methodology was different (wavelength interrogation technique where the wavelength is varied) than the one used here (angular interrogation technique where the

Figure 9.1 Schematic of the transfer process of graphene and WS₂ on a Au chip.¹²⁴

Figure 9.2 Schematic representation of the optical setup used for protein-protein interaction study.¹²⁴

wavelength of the light source is fixed). Additionally, all these earlier works were based on their self-designed customized scheme. Thus, in our current work, we make use of a commercial sensing platform that utilizes the Kretschmann configuration for assessing the plasmonic sensing capabilities of 2D materials. Figure 9.1 shows the transfer process of monolayer graphene and monolayer WS₂ on a Au chip.

The uniqueness of our work lies in the usage of a commercialized SPR system that has been used to test the efficacy of 2D materials for functionalization-free plasmonic bio-sensing. With this work, we intend to show a pathway for the generation of commercialized 2D nanomaterial coated Au chips that can be used in the future for plasmonic detection of biomolecules. Figure 9.2 illustrates our setup. Elaborating in brief, monolayer nanosheets of graphene and WS₂ coated Au chips are utilized for plasmon-based sensing of BSA and Anti-BSA interaction.

9.2. Simulation of graphene-WS₂ SPR structure

9.2.1. Angular Sensitivity Calculation

The sensitivity of an SPR sensor forms a crucial aspect as it dictates the ease by which biomolecules can be detected. Thus, in this section, with the help of simulation and using equations 4.1.1 to 4.1.7 we evaluate the angular sensitivity.

In case of Au only standard Kretschmann configuration, $N=3$ and for the heterolayered Au+graphene+WS₂ structure, $N=5$. The thickness of the Au layer is 50 nm, and for graphene and WS₂, we consider their thicknesses to be 1 nm each.

Figure 9.3a shows the basic SPR reflectivity curve at a wavelength of 785 nm. The dip in reflectivity is due to plasmonic resonance and the minima point in reflectivity marks the resonance condition where the wave vector of the incident light matches with the propagation constant of the surface plasmons. The graphene-WS₂ structure gives a 6.06% enhancement in sensitivity as compared to the Au only configuration (66 deg/RIU for Au only and 70 deg/RIU for Au+graphene+WS₂).

9.2.2 Electric Field Distribution

With the help of Comsol Multiphysics, we examine the normal component of the electric field at 785 nm. We consider the depth of the analyte region to be 1000 nm. This distance has been taken to clearly show the decaying nature of the evanescent field. It can be observed that the field decays to zero after it travels upto 400 nm. This is between one-half to one-third of the wavelength. Figure 9.3b displays the evanescent field variation as a function of the distance from the metal surface. In comparison to the standard Kretschmann configuration, the Au+graphene+WS₂ configuration can enhance the electric field at the surface by 7.89%. The penetration depth, L_p , which is defined as the distance at which the field becomes 1/e of its value at the metal surface, is improved by 2.77% for the heterolayer Au+graphene+WS₂ structure (114.98 nm) in comparison to the conventional structure (111.88 nm). An enhancement in the penetration depth improves the chances of the bio-molecular interactions to get detected.

9.3 Experiment Details

9.3.1 Consumables and instruments

Graphene and WS₂ were synthesized in a low-pressure CVD system inside a tube furnace (Lindberg/Blue M) equipped with a 1.2-meter wide and 1-inch diameter quartz pipe. For the bio-molecular experiment, SIA Kit Au (BR 1004-05), gold chips manufactured by GE Healthcare were utilized and the experiment was carried out using Biacore 3000.

9.3.2 Description of Biacore 3000 system

Biacore systems employ SPR methodology to assay real-time bio-molecular interactions that take place through the attachment of ligand molecules to the sensor chip that can capture and bind target molecules that flow through the analyte. The main system components comprise of the sensor chip, and the optical and the microfluidic systems. The sensor chip is made up of 50 nm gold and it is coated on a glass slide that is pressed against a semi-cylindrical glass prism. A near-infrared (NIR) light-emitting diode (LED) can excite the plasmons at the resonance condition. With the help of a delivery pump, the Integrated Microfluidic Cartridge (IFC) that has four flow channels and valves, supplies a continuous flow of the running buffer and the sample solution in a controlled fashion so that there is minimum disturbance and dispersion on the sensor surface.

9.3.3 Chemical Vapor Deposition Development of Monolayer graphene and WS₂

A single layer of graphene was fabricated on a Cu foil in the presence of hydrogen gas at 1030 °C flowing at a rate of 300 sccm for 90 min, which was then followed by a methane growth process at 1030 °C for 30 min at a flow rate of 10 sccm. Without changing the gas flow, the sample was rapidly cooled down to room temperature after it was removed from the heating zone of the furnace. WS₂ films were grown on a sapphire substrate using a similar setup. In a typical setup, 20 mg of tungsten (VI) chloride (WCl₆) powder (99.99%, Sigma-Aldrich) was kept at the middle of the furnace and 1 g of sulfur powder (Sigma-Aldrich) was kept at the upstream side. Sapphire substrates were kept downstream, 3–5 centimeters away from the middle of the furnace. Using high-purity Ar gas at a flow rate of 300 sccm, the furnace was purged for 5 min before the start of the heating process. The growth was conducted at 850 °C with a flow of 50 sccm

Figure 9.3: a) SPR reflectivity curve and b) Evanescent field distribution for the Au and Au + graphene + WS₂ layouts at 785 nm.¹²⁴

of Ar gas at a pressure of 2 Torr for 5 min. At the end of the synthesis process, the furnace was brought down to room temperature with the help of an electrically powered fan.

9.3.4 Transfer of CVD-Developed WS₂ and graphene

Firstly, the SIA Kit Au substrate was treated by Ar plasma (commercial 13.56 MHz RF source with power of 50 W, pressure of 40 Pa, 20 sccm Ar, 1min) before the graphene transfer process. The developed samples were spin-coated with a poly (methyl methacrylate) (PMMA) scaffold (5 wt % in anisole, 950 k molecular weight). The PMMA/graphene film was detached from the Cu substrate by etching it with 0.1 M (NH₄)₂S₂O₈. Alternatively, the PMMA/WS₂ film was detached from the sapphire substrate by etching it at 60 °C with 2 M KOH. For cleaning the detached films, they were carefully dipped into deionized water repeatedly. The PMMA/graphene film was then shifted onto the SIA Kit Au substrate and oven-baked at 120 °C for 10 min for sample attachment. Then, PMMA was removed by acetone. Subsequently, the PMMA/WS₂ film was moved onto graphene and oven-baked at 120 °C for 10 min. Finally, acetone was used to remove the residual PMMA. After the plasma treatment, the surface of the SIA Kit Au substrate becomes hydrophilic, which significantly enhances the interface bonding strength between graphene and the Au substrate. As for the interface of graphene and WS₂, they are monolayer 2D materials, which have a very strong Van der Waals' interaction. So, no shedding is observed after the preparation of the 2D material coated sensor chips. Also, the test area is mainly focused in the intermediate region. The exposed Au or partially covered graphene are at the edges of the SIA Kit Au substrate. This is due to the size mismatch of the graphene and Au films, and not due to shedding problems.

9.3.5 Experimental procedure for bio-molecular protein-protein interaction

SPR assays were performed on the Biacore 3000 (GE Healthcare) by using HEPES-buffered saline (HBS) pH 7.5 as a running buffer. Modified and mounted sensor chips were washed using distilled water before being docked into the Biacore instrument. Each sensor chip has four channels or flow cells Fc1, Fc2, Fc3, and Fc4 respectively. The experiment of protein-protein interaction was repeated four times. For a particular run, two of these flow channels were utilized. Streptavidin was used as a negative control protein and was coated on Fc1 or Fc3, which served as a reference flow cell. The active flow cells Fc2 and Fc4 were coated with BSA. Immobilization was carried out for 20 minutes at a flow rate of 5 μ L/min. Injected proteins were able to bind strongly to the modified sensor surface without additional coupling reagents. Following the immobilization procedure on each channel, the respective channel was blocked using 0.9 mM of Polyethylene Glycol (PEG) (MW 20,000) for 20 minutes. The flow rate was increased to 50 μ L/min. After blocking, the baseline in each flow cell was monitored for a few minutes to make sure there was no large drifts. Then, various concentrations of analyte Anti-BSA were flowed through both channels at the same time (Fc1 and Fc2/Fc3 and Fc4) for 1 minute. Several diluted concentrations ($\times 25$, $\times 50$, $\times 100$, $\times 200$, $\times 400$, $\times 800$ and $\times 1600$) of original stock solution of 0.7 mg/mL of Anti-BSA

were used. All dilutions were carried out using HBS buffer. The reference-subtracted data in the configurations (Fc2-Fc1) and (Fc4-Fc3) were obtained.

9.3.6 Characterization of graphene-WS₂ film

The as-obtained graphene-WS₂ heterostructures were examined by optical microscopy tools namely Olympus BX53M and RM (WITEC alpha 300R Confocal Raman system using a 532 nm laser as the excitation source).

9.4. Results and Discussion

9.4.1 Raman spectra and PL intensity

Graphene-WS₂ heterostructures were transferred onto the SIA Kit Au substrate (Fig. 9.4a). As shown in the enlarged optical image of the Au edge (Fig. 9.4b), we can see exposed Au, partially covered graphene/Au and the main fully covered WS₂/graphene/Au film. Fig. 9.4c shows a typical Raman spectrum at 532 nm excitation. The typical features of the spectrum are the monolayer WS₂ peaks located at ~ 350 and 418 cm^{-1} respectively that correspond to the E_{2g}^1 and A_{1g} modes. As for the Raman spectra of the heterostructure, the graphene and WS₂ peaks at $\sim 1585\text{ cm}^{-1}$ and $\sim 2680\text{ cm}^{-1}$ are indicated in the right of Fig. 9.3c. The intensity ratio of I_{2D} and I_G is approximately 2, and the full width at half maximum (FWHM) of the 2D peak is near 33 cm^{-1} , which confirm that there is a single layer of graphene. Fig. 9.4d shows the photoluminescence (PL) spectra of the monolayer WS₂ on a sapphire substrate and WS₂/graphene on the Au substrate. The PL spectra from WS₂ monolayer on graphene is remarkably extinguished due to the interlayer relaxation of the photogenerated excitons before recombination, caused due to the strong interlayer bonding between WS₂ and graphene. The above results confirm that we were successful in obtaining well-prepared graphene-WS₂ heterostructures, which are vital to the next step of SPR test.

9.4.2 Bio-molecular sensing of BSA and Anti-BSA interaction

We derivatized gold sensor chips with monolayer graphene and WS₂ and tested the binding of anti-BSA and BSA to these surfaces. In order to use this system to assay the interaction between BSA and anti-BSA, a reference surface has to be used to account for bulk effects and non-specific binding. However, we found that both anti-BSA and BSA bound strongly to the modified surfaces. We then chose Streptavidin, which presumably does not interact with either anti-BSA or BSA, to be coated on the reference surface. This ensures that anti-BSA does not bind excessively to the reference surface when injected.

The experiment was repeated four times. Since the flow cell in Biacore machine has four flow channels, for a particular experimental run, two out of these four channels were used. The sensorgram response is shown below in Figure 9.5. In brief, for the first set of operations, Streptavidin was first immobilized on flow channel Fc1. Figure 9.5a shows the corresponding sensorgram response. For the first two minutes of operation, baseline stability was established. The average response was 22780 RU. From time $t = 250$ seconds to $t = 1500$ seconds, the sensorgram response showed an exponential rise. The pattern is that of a typical protein immobilization. The curve shows the association and the steady-state phase. The

Figure 9.4: a) Photograph of graphene-WS₂ heterostructure transferred on the gold chip. b) Enlarged view of the dashed box at the edge of the gold chip in Fig 9.4a. c) Raman spectra of graphene-WS₂ heterostructure on the gold substrate. d) PL spectra of WS₂ monolayer on a sapphire substrate (red) and WS₂/graphene on an Au substrate (black), showing a significant PL quenching in WS₂/graphene.¹²⁴

response rose to 25900 RU at the end of immobilization. The dissociation phase was from $t = 1500$ seconds to $t = 1700$ seconds. The response dropped to 25800 RU during this phase. Following the immobilization phase, the surface of the sensor was blocked using PEG. Since this particular event was a non-binding operation, the response jumped to a higher value and fell back to the starting value at the end of twenty minutes. For the second set of operations, BSA was immobilized on flow channel Fc2. The sensorgram response is shown in Figure 9.5b. Again, baseline stability of 24450 RU was established for the first two minutes. After the immobilization, the sensor response was 25850 RU. The response dropped down to 25830 RU after the dissociation phase ended. Similar to the blocking operation in Fc1, the sensor surface was blocked using PEG. In the third set of operations, both the flow channels Fc1 and Fc2 were utilized. During this set, several diluted concentrations (0.44 $\mu\text{g/ml}$, 0.88 $\mu\text{g/ml}$, 1.76 $\mu\text{g/ml}$, 3.52 $\mu\text{g/ml}$, 7.04 $\mu\text{g/ml}$, 14.08 $\mu\text{g/ml}$ and 28.16 $\mu\text{g/ml}$) of Anti-BSA were passed over Fc1 and Fc2. Channel Fc1 acted as the reference here since it was immobilized with a non-related protein Streptavidin. Figure 9.5c shows the differential response (Fc1-Fc2). After the passage of each set of diluted concentrations of Anti-BSA, the baseline response was enhanced. The corresponding rises were 20 RU, 120 RU, 140 RU, 180 RU, 180

Figure 9.5 a. Sensorgram response for Streptavidin immobilization on Fc1 b. Sensorgram response for BSA immobilization on Fc2 c. Sensorgram differential response (Fc2-Fc1) for the flow of analyte Anti-BSA through Fc1 and Fc2.¹²⁴

RU, 190 RU and 190 RU for 0.44 $\mu\text{g/ml}$, 0.88 $\mu\text{g/ml}$, 1.76 $\mu\text{g/ml}$, 3.52 $\mu\text{g/ml}$, 7.04 $\mu\text{g/ml}$, 14.08 $\mu\text{g/ml}$ and 28.16 $\mu\text{g/ml}$ of Anti-BSA respectively. The enhancement in sensor response shows the binding activity between Anti-BSA and BSA. The Anti-BSA immobilization was done by preparing stock solutions using binary serial dilution. The highest concentration of Anti-BSA was diluted seven times to arrive at different diluted levels of the highest concentration. The immobilization of different concentrations of Anti-BSA was done in increasing order of the concentration levels. The sensorgram response showed that the incremental rise in baseline after the passage of every concentration level increased with enhanced concentration. The rise for 0.44 $\mu\text{g/ml}$ was 20 RU, which was a very small value. Further dilution of this concentration would not have given enough boost to the baseline and hence we concluded 0.44 $\mu\text{g/ml}$ to be a reasonable lowest limit that could be detected using the machine and the LOD of the Anti-BSA protein could be considered as 0.44 $\mu\text{g/ml}$.

Figure 9.6 shows the adsorption of BSA onto the graphene- WS_2 heterostructure. The white spots are BSA molecules that were adsorbed on the sensing surface. The background grey pattern is the heterostructure. The main purpose of the Scanning Electron Microscopy (SEM) image is to show the nanoscale dimension of the protein that interacts with the heterostructure.

9.4.3 Kinetic Analysis using Langmuir 1:1 Adsorption model

The Langmuir 1:1 Adsorption model describes the reaction kinetics between the ligand and the analyte. This is the simplest model that assumes a 1:1 interaction between the ligand and the analyte. We have used two association and dissociation data sets corresponding to the concentrations of 1.76 $\mu\text{g/ml}$ and 3.52 $\mu\text{g/ml}$ of Anti-BSA. The corresponding equations used are described below.¹²⁴

Figure 9.6a and 9.6b. SEM images of BSA adsorption on graphene-WS₂ heterostructure surface at different magnification scales.¹²⁴

$$A + B \rightleftharpoons AB \quad (9.4.3.1);$$

$$R_t = \frac{R_{max} [A]}{K_D + [A]} \left(1 - \frac{1}{e^{(k_a[A] + k_d)t}}\right) \quad (9.4.3.2);$$

$$R_t = R_o e^{-k_d t} \quad (9.4.3.3).$$

Describing the various variables, A and B are the analyte and ligand respectively. AB is the complex formed by ligand-analyte interaction. [A] is the molar concentration of analyte that is Anti-BSA in our case. k_a and k_d represent the association and dissociation constants for the complex formation process. R_t denotes the real-time response in RU. R_{max} is the maximum response in the association phase. R_o is the response at the end of association phase. Using the data from the two sets, we fit an exponential rise and decay curves for the association and dissociation phases with the help of the in-built curve-fitting tool in Matlab. The general model used for the two phases are $y = a \times (1 - \exp(-b \times x))$ and $y = a \times \exp(-b \times x)$ respectively. If we compare this with equations 9.4.3.2 and 9.4.3.3, the variables x and y represent t and R_t respectively. We arrive at the values of a and b and compare them with the corresponding terms in equations 9.4.3.2 and 9.4.3.3 to get the values of k_a and k_d . The various values that have been obtained are summarized below in Table 9.1. The average values of k_a and k_d are $2.765 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$ and $6.05 \times 10^{-5} \text{ s}^{-1}$ respectively.

Table 9.1. Tabular representation of kinetic analysis constants

Time Interval (in seconds)	1028.5 to 1093.5	1094.5 to 1197.5	1198.5 to 1269.5	1270.5 to 1362.5
Phase	Association	Dissociation	Association	Dissociation
Concentration of Anti-BSA	1.76 μg/ml		3.52 μg/ml	
Values of constants a and b	a = 403.5 b = 7.505×10^{-3}	a = 600.9 b = 6.32×10^{-5}	a = 302.1 b = 1.337×10^{-2}	a = 772.2 b = 5.78×10^{-5}
Values of k_a and k_d	$k_a = 2.92 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$ $k_d = 6.32 \times 10^{-5}$		$k_a = 2.61 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$ $k_d = 5.78 \times 10^{-5}$	

Table 9.2 compares the detection limits of other types of non-SPR sensors that have been used previously to detect BSA. The detection limit obtained with our device is 0.44 μg/mL is not that low, which is much better than the LOD values of any other non-SPR techniques listed in Table 9.2.

Table 9.2 Comparison of detection limits of BSA obtained here with other non-SPR sensors

S. No.	Type of sensor	Detection limit	Reference
1.	Side-hole fiber sensing	21 mg/mL	125
2.	UV-vis spectroscopy and X-Ray Fluorescence spectroscopy	1.3 µg/mL	126
3.	Memresistor	4 mg/mL	127
4.	Optical	0.2 mg/mL	128
5.	ELISA	5 µg/mL	129

9.5 Conclusion

Plasmonic biosensors are an interesting application of a relatively newly developed area of bio-physics called bio-photonics. Biomolecular protein-protein interactions modify the refractive index of the analyte and thus when these interactions occur within the evanescent field depth, it can modulate the output optical signal. The changing sensor response in the sensorgram thus provides relevant information on the interactions taking place in the analyte. In this work, we used monolayer nanosheets of graphene and WS₂ and coated them over gold chips for plasmonic detection of biomolecular coupling between BSA and anti-BSA. Using hydrophobic interactions, the protein structures can attach and immobilize themselves on the sensor substrate without the need for any functionalization methods that are usually required in conventional SPR sensors that use bare gold chips. In conclusion, this work can open up new scope in the area of using 2D nanomaterials for enhanced sensing performance of plasmonic biosensors with suitable pathological applications.

Chapter 10

Enhanced Goos-Hänchen shift plasmonic sensing with graphene

Abstract- Plasmon-based sensing relies on the interaction of photons with nano-structured materials. Surface plasmon resonance (SPR) biological and chemical sensors offer unique advantages of real-time and label-free detection techniques and hence they have been around in the commercial market for many decades. The principle of Goos-Hanchen (GH) shift, whereby linearly polarized light undergoes small lateral shift upon total internal reflection at a dielectric surface, is highly sensitive to refractive index (R.I.) changes in the sensing medium and can thus be utilized for plasmonic sensing. Two dimensional (2D) materials like graphene can boost plasmonic efficiency of these sensors because of their excellent optical properties. In this work, we have devised a customized GH shift-based SPR sensor using graphene-coated Au chips. With the help of experimental results, we show that graphene can enhance the sensitivity of the conventional Au only configuration by 2.35 times. Our custom-made GH shift optical setup can open up pathways to future research work where the sensing scheme can be optimized so that commercialization of the design can be gradually envisioned.

10.1 Introduction

2D materials are single-layer crystalline materials that have found innumerable applications in photovoltaics and electronics industry. The genesis of the 2D material industry began in 2004 with the invention of graphene. This unique material has phenomenal mechanical, electrical and optical properties and thus it has given ground-breaking results in many of its application areas. It is hundred fold stronger than steel and it also displays a current density that is 106 times that of Copper. Graphene has been utilized by many researchers in the field of optical sensors. Thus, graphene has long been researched employed in the field sensing. To be precise, when it comes to the main advantages of using graphene, it is its optical absorption in a broad optical spectrum of visible and near-infrared region of the electromagnetic light wave due to which the sensors can be operated at varying wavelengths in the given range, and its high mechanical toughness in terms of its tensile and compressive strengths because of which they can be incorporated in many kinds of flexible point-of-care (POC) devices. However, the low absorption of 2.3% can be a limiting point as well since many new 2D materials like transition metal dichalcogenides (TMDCs) and MXenes offer better absorption capabilities that can enhance the optical sensitivity to a much higher degree. In general, TMDCs provide high surface area due to which it is possible to have enhanced sensitivity. However, commercial manufacture of 2D materials still needs to be developed as the laboratory preparation method is not mature enough to be utilized on a large and systematic production platform. Additionally, the fabrication method is expensive and it is difficult to obtain a uniform thickness of the TMDC material. However, the fabrication technique of graphene is optimized as its production is not restricted to research laboratories. Hence, many commercial firms can synthesize high-quality graphene films that can be used for the development of high-performance bio-sensors. Additionally, many new kinds of materials like hybrid nanocomposite materials of polyaniline and carbon nano tubes (CNT), MoO₃, MXenes and V-V binary materials like Phosphorene and Antimonene have been utilized for many electronic and sensing applications. Materials like Phosphorene have been quite useful in optoelectronics because of their tunable bandgap properties due to which it can impart different functionalities to the device. Thus, 2D materials in general have a huge scope in the coming future where they will be incorporated in many nano-sized electronic and optics applications.

The GH shift is an optical phenomenon where the light beam that is reflected from the dielectric media interface shifts laterally from its geometrically predicted position. This technique has been applied in many areas of electronics, optical switching and sensing. Hence, this technique can be well integrated with the SPR method and a unique sensing scheme can be developed where the shift in the position of the reflected light can be used as an optical parameter to detect R.I. changes. As proposed by Artmann in the

stationary phase change method, the phenomenon of GH shift is closely related to the phase change of the reflected light. Mathematically, the GH shift can be obtained by differentiating the phase as a function

Figure 10.1 Schematic of the GH shift experiment setup denoting the various equipments used.¹³⁰

of the incident angle and is represent by equation 10.1.1 shown below where GH_{Sp} is the GH shift of the p -polarized light, λ is the wavelength, ϕ_p is the phase of the p -polarized reflected light and θ is the angle of incidence.¹³⁰

$$GH_{Sp} = -\frac{\lambda}{2\pi} \frac{d\phi_p}{d\theta} \quad (10.1.1).$$

In this present work, we make use of a customized setup to evaluate the effectiveness of graphene in enhancing the sensitivity of conventional Au-based plasmonic sensors. Figure 10.1 shows our setup. In brief, we use a 532 nm laser source and allow the light to pass through a focusing lens and a polarizer in order to get a p -polarized light that can excite the plasmons after it hits the BK-7 prism. The microfluidic system is used to insert and withdraw the analytes de-ionized (D.I.) water and 0.1 wt.% glycerol into the sample chamber. The GH shift in the position of the p -polarized reflected light is used as an optical output signal to assess the change in the R.I. of the sensing medium. A position sensitive detector is used to record the x-coordinate position of the reflected light. Since the reflected light changes its coordinate only in the x-direction and not in the perpendicular y-direction, we measure only the change in the x-coordinate position.

The main purpose of demonstrating this work is to show the utility of employing graphene for enhanced plasmonic sensing. Additionally, most commercial sensors employ angular interrogation technique. With this new customized setup of measuring the GH shift, we intend to introduce a new technique for measuring the R.I. change of the analyte. We observed that even with a small change in R.I. of 0.003413 (between D.I. water and 0.1 wt. % glycerol), the GH shift value in μm is quite large and thus our setup can be beneficial for detecting biomolecules that result in small R.I. changes of the sensing medium. The primary advantages of using graphene in any kind of optical sensing system has been highlighted above. In this specific scheme, the advantages of using graphene is in the mechanical toughness of the sensor film as the use of graphene can make the sensor surface scratch resistant. Additionally, as discussed above the sensing scheme with graphene can be used at varying wavelengths. However, the results in terms of sensitivity enhancement may vary. In terms of the drawbacks of this scheme, we are well aware that the sensitivity of the plasmonic sensor depends on the thickness and RI of the sensing layers. Since it is not possible to have low precision levels in terms of the thickness of the graphene layer, it is difficult to have a constant and uniform RI of the graphene layer at different batches of production and hence the optical sensitivity can vary. This is also reflected in our experimental results where we can see that

Figure 10.2 Schematic of the graphene transfer process on a Au chip.¹³⁰

different optical response in terms of GH shift is observed at different runs done on multiple graphene coated chips.

10.2 Experimental Method

10.2.1 Materials and Instruments

Materials: 50 nm thick Platypus Au chips coated on glass slide (Product Code: AU.0500.ALSI), BK7 right-angle glass prism, RI matching oil, sample chamber made out of polydiacetylene, microfluidic syringe and tube, de-ionized (D.I.) water, 0.1 wt% glycerol, Iso-propyl Alcohol (IPA).

Instruments: 532 nm laser source, polarizer, position sensitive detector, rotating turntable, Setup holder

10.2.2 Preparation of graphene coated Au chips

Graphene (G) film was grown using a low-pressure CVD system equipped with a tube furnace (1-inch, Lindberg/Blue M). Firstly, commercially available copper foil was electrochemically polished to obtain a clean and flatten surface. Then, the copper foil was loaded into the center of the tube furnace and annealed at 1030 °C for 90 min under a flow of 300 sccm hydrogen. After that, 10 sccm of methane was introduced for 30 min. After synthesis, the heating zone was rapidly cooled down to room temperature with the assistance of electric fans. Figure 10.2 shows a schematic of the graphene transfer process.

10.2.3 GH shift experiment setup

Figure 10.3 shows the various optical instruments that have been used to conduct the experiment. Additionally, it also shows the setup. A particular experimental run comprised of preparing the setup and consecutively taking the optical measurements. The top surface of the prism and the back-side of the Au/Au-graphene chip is first cleaned using IPA. The Au/Au-graphene chip was placed over the prism using the R.I. matching oil. The chip containing prism and the sample chamber were assembled together and fixed on the setup holder. The microfluidic tube was then attached onto the sample chamber from the backside of the prism. For the first set of experimental readings, D.I. water was injected into the sample chamber using the syringe.

The entire setup was mounted on the rotating turntable. The 532 nm laser light source is passed through a polarizer in order to get a *p*-polarized light source that is used to excite the plasmons. A position sensitive detector is used to measure the x_{position} of the reflected *p*-polarized light. The readings are collected by the position sensitive detector for 30 seconds and the x_{position} is calculated by taking the average of the collected readings. Thus, using D.I. water as the analyte, the x_{position} is measured for five different incident angles 72.92, 73.45, 73.97, 74.48 and 74.99 degrees respectively. The turn-table is rotated to increase the incident angle. After taking the measurements at 74.99 degrees, the D.I. water is replaced with 0.1 wt.% glycerol. With the new analyte, the x_{position} of the reflected light is measured in decreasing order of the incident angles. Thus, at a particular incident angle, the GH shift of the *p*-polarised reflected light can be calculated as shown below in equation 10.2.3.1. $x_{\text{position}}(Theta)|_{\text{D.I. water}}$ and $x_{\text{position}}(Theta)|_{0.1\% \text{glycerol}}$ are the $x_{\text{positions}}$ of the *p*-polarized reflected light at a particular incident angle Theta with D.I. water and 0.1% glycerol as the analytes respectively. $GH_p(Theta)$ is the GH shift of the *p*-polarized reflected light at angle Theta. The GH shift sensitivity *S* is as represented in equation 10.2.3.2 below and is given by the ratio of the absolute change in GH shift of the *p*-polarized

Figure 10.3 Optics experiment instruments and setup for the GH shift estimation of the Au and Au + graphene based SPR sensors.¹³⁰

reflected light to the change in R.I. of the analyte layer. For the bio-molecular sensing experiment, the experiment procedure remains the same except that we use Phosphate buffered saline (PBS) buffer and 0.25 mg/mL BSA in PBS as the analyte for the first and second sets respectively.¹³⁰

$$GH_p(\Theta) = x_{\text{position}}(\Theta) | 0.1\% \text{glycerol} - x_{\text{position}}(\Theta) | \text{D.I. water} \quad (10.2.3.1);$$

$$S = \frac{|\Delta GH_p(\Theta)|}{\Delta n_{\text{bio}}} \quad (10.2.3.2).$$

10.3 Results and Discussion

10.3.1 Characterization of graphene films

The as-grown graphene was transferred onto the Au substrate using the reported PMMA method. As shown in Figure 10.4a, graphene film covered most area of the Au substrate. The surface was clean and smooth, which was suitable for the SPR test. Meanwhile, the Raman spectra of the obtained graphene (Figure 10.4b) shows two obvious peaks (G band and 2G band) with the intensity ratio of I_{2D}/I_G about 2, which confirms that our graphene is monolayer. There is no peak around 1350 cm⁻¹ (D band), which means that our graphene is of high quality. In a word, we got the well-prepared graphene for the SPR test. The thickness of the graphene film is 1 nm and only a monolayer was coated on the Au chip.

10.3.2 GH shift enhancement calculation

For a particular type of chip (only Au and Au+graphene), the entire experiment was repeated five times. Thus, in total 10 sets of experiment readings have been obtained, five each for Au only and Au+graphene configuration. The experimental results for glycerol for all the ten runs have been summarized below in Table 10.1.

Figure 10.4 (a) Photograph of graphene film transferred on the gold chip. (b) Raman spectra of graphene on the gold surface.¹³⁰

Table 10.1 Summary of experimental results for glycerol as sensing molecule

Run	Incident Angle (in degree)	GH shift (in μm)	Average GH shift (in μm)	Standard Deviation (in μm)	Max. GH shift (in μm)	Min. GH shift (in μm)	Average Enhance ment	Max. Enhancement	Min. Enhancement
Au									
1	74.48	37.67	35.42	9.14	44.56	26.27	2.35	3.84	1.47
2	73.97	23.59							
3	73.45	43.06							
4	73.97	44.42							
5	72.92	28.36							
Au and graphene									
1	74.48	81.85	83.31	17.71	101.02	65.59			
2	73.45	75.75							
3	73.45	60.39							
4	72.92	90.35							
5	73.97	108.23							

Though for every run, the GH shift measurements were taken for all five incident angles, in the table above only the maximum GH shift and the corresponding incident angle has been represented. The average GH shift has been arrived by taking the average of the five runs. We could get an enhancement of 2.35 for the Au + graphene layout as compared to the standard Au only Krestschmann configuration. The maximum and minimum GH shift values for a particular scheme have been arrived by adding and subtracting the standard deviation value to the average value respectively. For the Au only layout, the range of the GH shift value is from 26.27 μm to 44.56 μm . And for the Au + graphene configuration, the corresponding range is from 65.59 μm to 101.02 μm . Since the two ranges do not overlap and are well placed apart, we can confidently say that the Au+graphene layout gives better GH shift values as compared to the Kretschmann configuration. The maximum enhancement value has been arrived by considering the ratio between the maximum GH shift value for the Au+graphene layout and the minimum GH shift value for the Au only configuration. On similar terms, the minimum enhancement is the ratio between the minimum GH shift for the Au+graphene scheme and the maximum GH shift for the Au only layout.

For the bio-sensing experiment, a total of six experiment runs were performed (three each for Au and Au + graphene chips). The experiment results have been tabulated in Table 10.2. The average

Table 10.2 Summary of experimental results for BSA as sensing molecule

Run	Incident Angle (in degree)	GH shift (in μm)	Average GH shift (in μm)	Standard Deviation (in μm)	Max. GH shift (in μm)	Min. GH shift (in μm)	Average Enhancement	Max. Enhancement	Min. Enhancement
Au									
1	73.45	36.79	62.54	45.99	108.54	16.54	1.69	8.26	0.69
2	73.97	35.19							
3	73.97	115.65							
Au and graphene									
1	73.97	141.05	105.93	30.79	136.72	75.13			
2	74.48	83.56							
3	74.99	93.18							

enhancement is 1.69 and the maximum and minimum enhancements are 8.26 and 0.69 respectively. An enhancement value less than one implies that the GH shift range for Au and Au + graphene overlap. This is because of the relatively high variability obtained in this set of biomolecule sensing experiment as compared to the basic test using glycerol. The reason can be attributed to many factors like the quality of the manufactured batch of graphene, compactness and tightness of the optical setup as the presence of slight contamination or even air-gaps can alter the results to a great extent. However, the results still corroborate our premise of being able to perform bio-molecular sensing using the GH shift method. In addition to these tests, we also performed an experiment where we measured the GH shift as a function of increasing concentration of BSA. The experiment was carried out at an incident angle of 73.97 degree. The response for D.I. water was first measured and then increasing concentrations of BSA were allowed to flow successively through the sensor and the corresponding GH shifts were calculated. Figure 10.5 shows the response for eight different concentrations 7.8125 $\mu g/mL$, 15.625 $\mu g/mL$, 31.25 $\mu g/mL$, 62.5 $\mu g/mL$, 125 $\mu g/mL$, 250 $\mu g/mL$, 500 $\mu g/mL$ and 1000 $\mu g/mL$ of BSA respectively. We observe an exponential rise of the curve where the response is linear for lower values of concentration and it saturates at higher concentration. This provides an useful insight in terms that the GH shift method can be employed for the detection of low concentrations of small biomolecules.

Figure 10.5 GH shift response for varying concentrations of BSA for the Au + graphene layout.¹³⁰

10.4 Conclusion

The field of bio-sensing is no longer limited to a single discipline but involves multiple domains like nanotechnology, physical and biological sciences. Plasmonic sensing is an optics-based detection technique that relies on the change in R.I. of the analyte layer for the detection of biomolecules or chemical species. SPR sensors use various kinds of interrogation techniques like angular, phase and wavelength interrogation. The phenomenon of GH shift can also be employed to detect the change in R.I. of the analyte layer due to the presence of molecules of interest. Thus, in this work, we developed a custom-made system that functions at a wavelength of 532 nm, comprising of graphene coated Au chips, for the enhancement of GH shift sensitivity as compared to the standard Kretschmann configuration. The graphene coated Au chip configuration gives an average GH shift of 83.31 μm as compared to 36.22 μm for the standard Au only Kretschmann configuration, thus resulting in a 2.35-fold sensitivity enhancement.

Chapter 11

Conclusions and suggestions

Plasmons are a result of EM light waves interacting with the free electrons present on the surface of noble metals like Gold and Silver. We started with the chapter on thesis overview where we talked about the concept of surface plasmons (SPs), SPPs, SPR sensing, LSPR sensing and the application of plasmonic sensing in bio-medicine especially POC testing.

Next, we proceeded to have a thorough introduction on the physics behind SPP and other concepts of plasmonic sensing with an emphasis on the mathematical side that highlight the nature of the SPs with the help of governing equations. As said in the abstract, the thesis mainly revolves on the aspect of using low dimensional materials for plasmonic sensing and this has been divided into two major parts. The first part is about using simulation studies to develop and evaluate the performance of SPR models and the second part is on verifying the actual capability of 2D materials in enhancing the sensitivity of SPR sensors and we do this by performing biological and optical experiments.

Thus, before describing the actual research work, we perform a literature review where we look at some of the models that have been earlier proposed by researchers with the help of simulation. We also take a look at some of the biological experimental works that have been done in the area of plasmonic sensing with the help of 2D material based SPR sensing. Moving further, part 1 basically comprises of chapters 4 through 7 where in chapters 4 and 5 we focus on using simulation as a tool to predict the best desired SPR sensing arrangement. Chapter 4 uses low dimension titania and silica for the modification of conventional Kretschmann configuration and chapter 5 uses various combinations of ARCs - TiO_2 , SiO_2 , Nb_2O_5 and MgF_2 along with different TMDC materials - MoS_2 , WS_2 , MoSe_2 and WSe_2 . In chapters 6 and 7, we propose a plasmonic sensing arrangement for the detection of the SARS-CoV-2 virus and with the help of simulation show that SPR sensing can be quite promising.

Coming to the experimental part 2, this comprises of chapters 8 through 10. In chapter 8, we evaluate the plasmonic sensing capability of the 2D materials MoS_2 , WS_2 and graphene with the help of a commercial SPR machine and this lays the groundwork for the rest of the experimental work. We arrived at the best sensing configuration and used it for the study of bio-molecular interaction of proteins and found that 2D materials can be highly useful for functionalization-free sensing and the details have been discussed in chapter 9. We end with chapter 10 by discussing about the GH-shift experiment done to show the enhanced plasmonic sensing abilities of graphene coated Au chips.

Thus, to conclude we were able to show the effectiveness of 2D materials in boosting the sensitivity of conventional SPR arrangement. We showed that 2D materials can be highly useful for functionalization-free sensing as they can directly attach the ligand molecules with the help of hydrophobic interactions. Other properties of 2D materials like high surface area to volume ratio enhances the capture of antibodies. With that a lot of work can be done in this regard where we could have tested whether 2D material coated chips could be used for the detection of other protein molecules like specific antibodies Tumor Necrosis Factor Alpha ($\text{TNF-}\alpha$) or other molecules like immunoglobulins, human growth hormone etc. The aspect of commercialization of 2D material coated chips can be a good direction for further work. At present, most of the 2D materials like MoS_2 , WS_2 , PtSe_2 , h-BN, MoO_3 , MXenes etc. are laboratory produced and thus to be able to synthesize commercial bio-sensing chips coated with these materials will require lot of efforts in fine-tuning and optimizing the fabrication procedure to enhance the durability and stability of such chips.

We performed some basic GH-shift sensing using graphene coated chips. We used a customized set up to take the measurements. GH-shift sensing can be highly beneficial for the detection of low concentrations of bio-molecules and thus a systematic and detailed work in this direction can also prove to be enlightening where the mechanical design of the optical setup can be optimized to make it more robust and take it to a commercial level.

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Summary

To summarize the entire thesis, in chapter 2 we focused on introducing the basic concept of SPR, the meaning and relevance of the term sensitivity and on ways to enhance it. Moving ahead, in chapter 3 we briefly reviewed some literature where we saw how various researchers used simulation to design hybrid SPR structures and also used 2D materials for performing bio-molecular sensing experiments.

Further, in our actual work, we divided it into two main parts. The first part consists of simulation. In chapter 4, we designed a SPR sensor using TiO_2 and SiO_2 . We performed the simulation at various wavelengths of 532 nm, 600 nm, 633 nm and 785 nm and found out the optimized thicknesses at each wavelength. We found that the best sensitivity result of 214 deg/RIU is obtained at a wavelength of 532 nm with thicknesses of 40 nm Au, 3 nm SiO_2 and 9 nm TiO_2 . In chapter 5, we again designed a more complex scheme using ARCs and TMDCs and performed the simulation at 633 nm and saw the effect of having ARCs in the SPR structure. To conclude, the combination of 50 nm Au, 95 nm of TiO_2 , 20 nm of SiO_2 and 2 layers of 0.7 nm each of WSe_2 gave the best sensitivity of 284 deg/RIU. Taking our simulation work a bit further, in chapter 6 we proposed a plasmonic immunosensing assay where we provided a theoretical structure to detect the COVID-19 virus. Taking another step ahead, in chapter 7 we used Au NRs and proposed a sandwich type plasmonic assay for detecting SARS-CoV-2 virus.

Moving ahead, in the second part we focus on bio-molecular and optical sensing experiments. To begin with, in chapter 8 we discuss about how we conducted a basic bio-molecular test with the help of a commercial SPR sensor Biacore 3000. We evaluated the efficacy of 2D materials graphene, WS_2 and MoS_2 in plasmonic sensing. We found that the sensitivity in increasing order is as follows: only Au < Au+ MoS_2 < Au+ WS_2 $\approx$ Au+graphene < Au+graphene+ MoS_2 < Au+graphene+ WS_2 . In chapter 9, with the help of a heterolayered SPR structure based on WS_2 coated over graphene, we performed a protein-protein interaction study using Biacore 3000. We could realize a functionalization-free detection scheme where using graphene- WS_2 we could detect 0.44 $\mu\text{g/ml}$ of Anti-BSA. Finally, we concluded our research work with an optics-based experiment where using a customized setup we evaluate the sensitivity of graphene coated Au chips by measuring the GH shift as the optical output or response. We found that the Au + graphene plasmonic sensing configuration gave 2.35 times enhancement as compared to the standard Kretschmann configuration.

Conclusion

Plasmonic bio-sensors offer unique advantages over other sensors based on different methodologies like real-time, label-free detection, reusability of sensor chips and high accuracy results that are repeatable in nature. These sensors work on the basis of basic principle of SPR which is a physical phenomenon that occurs with the interaction between EM light wave and nano-sized plasmonic metals like Au and Ag.

Bio-molecular interactions that occur at the sensor surface have the ability to change the R.I. of the analyte medium. The change in this parameter produces a change in the optical parameter like the resonance angle, the phase shift of the reflected *p*-polarised light w.r.t. the *s*-polarised light or GH shift between *p* and *s* polarised reflected light waves. Thus, a change in these optical parameters can be used to detect bio-molecular interactions occurring at the sensor surface.

The sensitivity of these SPR sensors play an important role in the precision and accuracy of detection of bio-molecular ligand-analyte or protein-protein interactions. Sensitivity in general can be defined as the differential change in optical parameter viz. the SPR angle, phase or GH shift w.r.t the change in the R.I. of the analyte medium. Conventional sensors employ the Kretschmann configuration which is 50 nm Au coated on a high R.I. prism.

In the first one and half year of the research, we focused on the use of simulation methods in predicting the sensitivity of SPR structures made of hybrid multi-layer low dimensional materials. We particularly focused on ARC materials like titania and silica and TMDC materials like WS₂, MoS₂, WSe₂ and MoSe₂. The purpose of the simulation study is to know how it can help in the preliminary assessment of the sensing capabilities of a SPR structure. Simulation can never give an actual estimate of the effectivity of the designed structure as the performance of the sensor depends on various factors like the quality and uniformity in the thickness of the synthesized nanomaterial, the actual RI of the layers and experimental conditions like proper alignment of the laser source, tightness in the SPR structure. However, simulation study will always be useful as it can help in the comparison of effectiveness of different SPR arrangements.

Taking our work further, in the next one year we focused on bio-molecular and optics experiment. In the biological sensing experiment, we performed two main experiments, where first we did a basic plasmonic sensing comparison of various 2D materials with the help of a commercial SPR machine. In the second experiment, we performed a protein-protein interaction study between Anti-BSA and BSA by graphene and WS₂ modified gold chips. In the optics experiment, we compared Au and Au + graphene based SPR configuration by evaluating the GH shift response of both the arrangements.

Thus, to conclude our entire work, we first used simulation to analyze how the thickness and RI of every low dimension layer affects the sensitivity. With the help of bio-molecular and optics experiments, we confirmed that 2D materials can actually be effective in enhancing the sensitivity of plasmonic arrangements.

As a future work, a lot can be done in the coming years. For instance, researchers can work on the commercialization of 2D material coated chips. The technique used here is not optimized and benchmarked for the purpose of clinical use. Hence, this is one area that will require a joint effort from the medicine, fabrication industry and academic research firms. Additionally, for the optics experiment we used our own developed setup that can be further improved in terms of complexity and practical usage.

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