3D- Printed Modular SPR Platform Integrated with Ellipsometer for In-situ Label-free Optical Sensing Applications

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Abstract

We present the instrumentation of highly sensitive, label-free and nondestructive Surface Plasmon Resonance-enhanced Ellipsometry (SPRE) technique using the efficient 3D printing technology and its applications for the sensitive detection of biochemical analytes. SPRE offers the advantages of both SPR and Ellipsometry while eliminating the limitations encountered with each individual method. 
3D-Printed Modular SPR Platform Integrated with Ellipsometer for In-situ Label-free Optical Sensing Applications

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Abstract- In this study, we investigate the integration of surface plasmon resonance (SPR) spectroscopy with a commercial ellipsometer using a customized 3D-printed module to achieve a highly sensitive, label-free optical sensing platform. Our in-house built SPR module's simple design circumvents the optical alignment's complexity while integrating with the ellipsometer. 3D printing technology enables the construction of customized SPR module with a flow-cell, which is a crucial component for its operation in liquid media. The performance of the developed SPRE platform is demonstrated through label-free optical sensing. The refractive index sensitivities for wavelength and phase interrogation were found to be $3106\pm171$ nm/RIU and $5842\pm288$ °/RIU. We have demonstrated the sensor's response to the adsorption of multilayer poly-electrolyte molecules. The protein-protein (BSA/Anti-BSA) interactions were also performed, and a detection limit of 5.2 pM was achieved for the Anti-BSA detection. We believe that our present work will widely open the functionality of SPRE sensors with enhanced sensitivity, rich information, and high precision by enabling the advantages of both SPR and Ellipsometry techniques. Furthermore, this study will serve as a foundational framework for researchers, shedding light on the potential of 3D printers in laboratories and expediting the development of cost-effective integrated sensing platforms.

Index Terms- Ellipsometry, SPR, 3D printing, Optical Sensor, SPR-enhanced Ellipsometry, Biosensor.

I. INTRODUCTION

In recent years, optical sensors based on reflectance, transmittance, absorbance, and fluorescence have been employed widely due to their simpler configuration, stability, cost-effectiveness, rapid data acquisition, high sensitivity, and lower detection limit [1]–[4]. Ellipsometry and Surface Plasmon Resonance (SPR) spectroscopy-based optical sensing techniques are both popular due to their higher sensitivity, label-free, real-time, high throughput, simpler instrumentation, and non-destructive nature. With the rising demand for optical sensors, the integration of SPR and in-situ ellipsometry enables the advantages of both SPR and Ellipsometry for the sensitive detection of biochemical analytes with high precision by avoiding the limitations of each technique [5]–[8]. Spectroscopic Ellipsometry (SE) is a well-known, versatile, indirect, non-destructive, and powerful optical characterization technique that has been evolved from research laboratory to industrial applications for determining thin film thickness and optical constants of different materials [9]. Since the '90s, the application of SE has been employed in a wider range due to its commercialization instruments, advanced data analysis method, extended characterization from semiconductors to biomaterials, and optically anisotropic material [10]–[12]. Ellipsometry is most often utilized in external reflection mode and enables real-time monitoring of molecular interactions [13]–[15]. Since SE signal is self-referencing, baseline signal correction is not required, which reduces the light source noise and ensures accurate signal measurement. As the ellipsometry measurement is independent of...
light intensity, the change in polarization states is more informative to analyze the phase, which shows significant change close to the SPR condition. Significant limitations encountered with SE are that the measurements require smaller surface roughness of samples and oblique incidence of light probe [13]. Ellipsometry measurement is restricted at normal incidence because p- and s- polarizations can not be differentiated at this angle. These limitations can be overcome when ellipsometry is operated in the total internal reflection (TIR) mode of SPR Kretschmann configuration, known as Total Internal Reflection Ellipsometry (TIRE) or Surface Plasmon Resonance Ellipsometry (SPRE) [16]–[20]. Ellipsometry measurements under TIR condition exhibit enhanced sensitivity for thin films when operated close to SPR condition in Kretschman configuration. In SPR sensing, the evanescent field generated by surface plasmons interacts with the analyte media [21]–[23]. Therefore, the direct interaction of light with the sensor's surface can be avoided using the SPRE sensing platform, allowing measurements in opaque or semi-transparent media, a limitation of conventional SE measurements [16]. The SPRE sensing platform offers to implement both the amplitude (intensity) and phase interrogation, which are limited to the conventional SPR system. Nabok et al. [24] have experimentally investigated that the SPRE technique is more accurate and sensitive than SPR by detecting low molecular weight hazardous environmental toxins. The protein binding study has been reported earlier by using SPRE platform [18], [25], [26]. Wu et al. [27] have reported the ability of SPRE for the sensitive and rapid diagnosis of Malaria based on malaria-specific plasmodium parasite detection with a 12 pM limit of detection (LOD). The potential of SPRE for investigating the metal-oxide nanostructures modified complex substrate for protein binding study with enhanced sensitivity has also been reported by Plikusiene et al. [28]. Fischer et al. [29] have also investigated the potential of the SPRE technique for gas sensing by the detection of COH₂ and C₃H₆ with a 10 ppm LOD. M. Poksinski et al. [30] have investigated copper corrosion using the SPRE.

Although SPR and SE techniques originated decades earlier and are present at an advanced stage, the advantages of the SPRE technique are yet to be explored. Compared with SPR, SE is more expensive but yields more information about the sample under investigation. Nowadays, the development of integrated sensing platforms is much desirable due to their high sensitivity, selective detection, high efficiency, reproductibility, precise analysis, and reliability. A single system requires proficient instrumentation to integrate two or multiple sensing techniques. The commercially purchased device or any relevant components of the experimental set-up for the instrumentation are quite costly, and sometimes, time-consuming techniques or expensive materials are required. The 3D (three-dimensional) printing technologies have recently become feasible for manufacturing complex components and structures [31]. Additive manufacturing or 3D printing technologies enable the design and fabrication of intricate geometric objects and an economical method of constructing complicated objects for research and biomedical applications. The widespread adoption of 3D printers and their user-friendly software has significantly expanded their use in advanced technological fields [32]–[34]. Yet the use of 3D printers for instrumentation applications and apparatus in research laboratories has not been explored much due to the lack of fundamental documentation for the scientific community [35]. Our present study focuses on the instrumentation of SPRE platform by developing a compact, user-friendly, light-weight, and durable 3D-printed SPR module, which is incorporated smoothly with the commercial ellipsometer. Using this simple configuration of our custom-built SPR module, we can combine SPR and ellipsometry, two powerful optical analytical techniques, in a single sensing platform at low cost. Our proposed module eliminates the difficulty in optical alignment required to integrate with ellipsometry. The performance of our developed set-up has been demonstrated by the sensitive detection of biochemical species and the multilayer adsorption of polyelectrolytes. We achieved bulk refractive index sensitivity around 5842±288 °RIU (Δ sensitivity) for ellipsometry phase modulation and LOD of 5.2 pm for the analyte Anti-BSA detection using a conventional Au sensor chip. Our custom-built SPR-module offers a highly sensitive, label-free, and efficient integrated optical sensing platform for different biochemical sensing applications in forthcoming times. The main focus of our present work is the SPRE instrumentation and to address the gap between scientific researchers and 3D printing technology by constructing commercially compatible devices or components cost-effectively with less time for custom-made unique designs. Further, the 3D printed device has been incorporated to integrate SPR with ellipsometry for developing a highly sensitive optical sensing platform. To the best of our knowledge, there is no earlier report of manufacturing such a unique, compact, and cost-effective 3D-printed SPR module that has been explicitly designed for label-free and efficient SPR sensing.

II. THEORETICAL BACKGROUND

Paul Drude first invented the manually operated ellipsometry in 1887 [36], and later on, Aspnes developed the completely automated SE in 1975 [37]. Muller and Farmer first reported the real-time monitoring of SE in 1984 [38]. Ellipsometry measures the change in polarization states in terms of Psi (Ψ) and
\[\Delta \text{ parameters after incident light gets reflected from the sample's surface.} \]

The schematic in Fig. 1 shows the basic principle of ellipsometry measurement. The mathematical expression for the measured signal of ellipsometry is defined by the following equation [13]:

\[
\rho \left( \tilde{n}_0, \tilde{n}_1, \tilde{n}_2, d, \theta_i \right) \equiv \frac{r_p}{r_s} \equiv \tan \Psi, e^{i\Delta} \quad (1)
\]

\[
\tan \Psi = \frac{|r_p|}{|r_s|}; \Delta = \delta_p - \delta_s \quad (2)
\]

Where \(\tilde{n}_0, \tilde{n}_1, \tilde{n}_2\) are the complex refractive indices of the ambient (\(\tilde{n}_0 = 1\)), a thin film with thickness \(d\) and substrate, respectively. Instead of light reflection, if the transmission is considered, then \(\rho\) will be the ratio of Fresnel's transmission coefficients \(\rho \equiv \frac{t_p}{t_s}\), \(r_p\) and \(r_s\) represent the Fresnel's complex amplitude reflection coefficients for p- and s-polarized light. And can be expressed as,

\[
r_p = \frac{E_{rp}}{E_{ip}} = |r_p| \exp (i\delta_{rp}) \quad (3)
\]

\[
r_s = \frac{E_{rs}}{E_{is}} = |r_s| \exp (i\delta_{rs}) \quad (4)
\]

\[
t_p = \frac{E_{tp}}{E_{ip}} = |t_p| \exp (i\delta_{tp}) \quad (5)
\]

\[
t_s = \frac{E_{ts}}{E_{is}} = |t_s| \exp (i\delta_{ts}) \quad (6)
\]

At total internal reflection condition, the Fresnel complex reflection coefficients \(r_p, r_s\) for p- and s-polarized waves can be written as [16],

\[
r_p = \frac{n_1 \cos \Phi_0 - n_0 \cos \Phi_1}{n_1 \cos \Phi_0 + n_0 \cos \Phi_1} \quad (7)
\]

\[
r_s = \frac{n_0 \cos \Phi_0 - n_1 \cos \Phi_1}{n_0 \cos \Phi_0 + n_1 \cos \Phi_1} \quad (8)
\]

Where \(\Phi_0\) and \(\Phi_1\) represent the incident angle and angle of refraction at the interface of two different media having refractive indices of \(\tilde{n}_0\) (\(n_0\)-ik\(_0\)) (denser media) and \(\tilde{n}_1\) (\(n_1\)-ik\(_1\)) (rarer media).

The refractive index is a function of wavelength \(\lambda\). The range of Ellipsometry parameters \(\Psi, \Delta\) is defined in the range of \(0<\Psi<90^\circ\) and \(-180^\circ<\Delta<+180\) or \(0<\Delta<360^\circ\), respectively. The term "internal reflection" signifies that the incident light is reflected at the interface when the refractive index of the incoming media is greater than the reflecting media. In this approach, the incoming semi-infinite medium is considered transparent and generally comprised of glass. The reflecting semi-infinite media is dielectric (gas or liquid) of complex refractive index. In TIRE configuration, \(r_p \approx \tan^2 \Psi\) and \(r_s\) is close to 1. And \(r_p\) or \(\Psi\) spectra become minimum, and simultaneously, \(\Delta\) spectra show a steep fall. A software-generated optical model is built to interpret the measured signal and different parameters, i.e., film thickness, surface roughness, and optical constants, are extracted.

III. INTEGRATION OF SPR MODULAR UNIT WITH COMMERCIAL ELLIPSMETER

The 3D-printing technology has been adapted to transform our customized 3D model into a physical object by layer-by-layer construction using a non-reactive polymer material at a low cost. As the SPR concept is based on the SPR Kretschman configuration, the 3D SPR module was designed in such a way that its simple configuration could eliminate the challenges in optical alignment while integrating with the ellipsometer. Based on our experimental requirements, the 3D animated model shown in Fig. 3a was first designed using TinkerCad software (freely available online). The 3D printer incorporates a heated nozzle of 0.4 mm diameter through which the precursor polymer is fed. PVA polymer was used to print the 3D model. The melted polymer is released from the nozzle, and the desired design is fabricated on a flat hotbed. The position is controlled by using the X/Y motors. The sensor chip is placed in the middle of the prism (using a small drop of index-matching oil) and incorporated with a flow-cell to introduce the analyte. Once the pattern for one layer has been deposited, the stage is lowered by a predetermined layer height, facilitating the deposit of additional layers along the Z-axis. As the polymer melts, it binds to the previous layer to produce the 3D model with uniform consistency. The printing precision of our 3D printer is ±0.1mm. The digital image of the
separate parts of the printed SPR module and the complete set-up are shown in Fig.3 (b,c).

**Fig. 2.** Schematic representation of SPRE configuration used for the experimental set-up.

The developed SPR module is user-friendly and has a unique design with features of light-weight, durability, and cost-effectiveness. The material used for printing the module is non-reactive; thus, there is no possibility of contamination, and it could be easily cleaned. Our customized SPR module is divided into two parts, e.g., one portion consists of the dove prism and two holes aligned in line with the prism for passing incident and reflected light and another detachable portion consists of the flow cell (inlet/outlet channel) for passing analyte of interest and locking system. The dimension of the dove prism holder is (10 cm × 4.1 cm × 3 cm).

**Fig. 3.** (a) Design of the 3D model for building SPR cell set-up using CAD software. (b,c) Digital image of the complete SPR module assisted with a dove prism after building from a 3D printer. (d) Digital image of the integrated SPRE set-up where a 3D-printed SPR cell is mounted on a commercial ellipsometer.

The sensor chip is placed within the central region of the prism by introducing a small amount of index-matching oil. A silicon O-ring (1 cm²) is used while connecting the flow-cell (Fig.3c) to avoid any possible leakage of the analyte. The analyte could be flown over the sensor’s surface using a syringe pump connected to the inlet/outlet flow channels, as depicted in Fig.3d.

After developing the SPR module, it was mounted on the sample stage of the commercial Ellipsometer (Alpha SE, J. A. Woollam, wavelength range of 380-900nm) in in-situ mode (at 90°). The successful replication of the SPR Kretschmann configuration through this alignment of the module enables the seamless integration of SPR and ellipsometry. After successful integration, all the experiments were performed by using our in-house-built SPR module, as depicted in Fig.3, at room temperature (27°C) and +10 s data acquisition rate in the in-situ mode.

IV. EXPERIMENTAL SECTION

A. Chemical Reagents and Materials

Poly-diallyl dimethylammonium chloride (PDAA) (Sigma-Aldrich), poly(sodium styrene sulfonate) (PSS) (Sigma-Aldrich), glycerol (Merck), 11-mercapto undecanoic acid (MUA, Sigma-Aldrich), 1-(3 Dimethylaminopropyl)-3-Ethyl Carbodiimide Hydrochloride (EDC.HCl, CaH2N3.HCl, Sigma Aldrich), N-Hydroxyssuccinimide (NHS, Sigma Aldrich), DI water (Millipore, Merck), Bovine Serum Albumin (BSA, Sigma-Aldrich) and Anti-Bovine Serum Albumin (Anti-BSA Sigma-Aldrich), 1X Phosphate Buffer Saline (PBS, pH 7.1, Sigma-Aldrich), Absolute ethanol (Sigma-Aldrich), Acetone (Alfa-Aesar), Isopropanol (Sigma-Aldrich), micro-glass slides (Riviera, India), Index-matching oil (Merck), BK7 dove prism (Thor lab, USA). All chemicals were used without further purification.

B. Sensor Chip Preparation and Surface Modification

The glass substrate with a thin film of Cr/Au is utilized as a sensor chip due to the chemical inertness, high sensitivity, and biocompatibility of Au. The sensor chip was prepared by depositing 4 nm Cr and followed by 42 nm Au thin film (pressure 6×10⁻⁶ Torr, rate 0.2 Å/s) on a pre-cleaned glass substrate using electron beam evaporation. Before deposition, the glass substrates (3cm × 2 cm), were cleaned using ultrasonication in Piranha solution (3:1 ratio of H₂SO₄ and H₂O₂). Further, the glass substrates were ultrasonically cleaned with DI water, acetone, and ethanol, respectively, and finally, after drying, kept in a moisture-free environment until the Au deposition. For protein sensing experiments, the Au sensor chip was functionalized with 11-mercaptoundecanoic acid (MUA) and followed by 1-(3-Dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (EDC), N-Hydroxysuccinimide (NHS) amine-coupling chemistry for specific binding of protein molecules. To functionalize the sensor's surface, it was initially immersed in a 2 mM 11-MUA solution.
(prepared in ethanol) overnight to form a self-assembled monolayer (SAM) of the alkanethiol group. After removing from the MUA solution, the chip was cleaned in ethanol to remove excess unbound molecules and dried by heating at 120°C for 1 h. The sensor chip was further dipped into the EDC-NHS (5mM:2mM ratio, prepared in DI water) solution to activate the carboxylic groups for 6 h. After removing from the solution, the sensor chip was rinsed with DI water and heated at 120°C for 1 h for strong binding with the linker groups.

C. Bulk Refractive Index Sensing

Prior to proceeding with the biosensing application, the bulk refractive index (RI) sensitivity test was performed using 0-25% concentrations of glycerol-water solutions having refractive indices of 1.3330, 1.3388, 1.3448, 1.3496, 1.3572, and 1.3624 [39]. The Ellipsometry signal was recorded in terms of Ψ, Δ while the Au sensor chip was exposed to air, water and glycerol-water solutions. The spectroscopic and real-time responses were recorded by sequentially passing the 0-25% concentrations of glycerol-water solutions over the Au sensor chip through the flow cell controlled by a syringe pump at a flow rate of 1ml/h for 10 mins.

D. Detection of Chemical and Biological Species using SPRE

After determining the bulk refractive index sensitivity, the integrated system has been used for label-free detection of chemical and biological analytes using polyelectrolytes and proteins. At first, we initiated our investigation to detect the interactions between cationic and anionic polyelectrolytes to mimic the label-free sensing ability of our developed SPR ellipsometry sensor. In the experiment, PDDA was used as a cationic polyelectrolyte and poly PSS as anionic polyelectrolyte. Both the electrolyte solutions were prepared in DI water with a 1mg/ml concentration. At first, the PDDA solution was introduced to the Au sensor chip through the flow cell with a flow rate of 1ml/hr for 10 minutes. During the flow of the PDDA solution, the interaction between PDDA and the Au surface was monitored by the change in Ψ and Δ signals with time. The sensor chip was exposed to PSS solution by following the above flow rate and timing. The interaction between PDDA and PSS resulted in a gradual shift in both the Ψ and Δ signals. The experiment was performed for layer-by-layer interactions between cationic PDDA and anionic PSS electrolytes and repeated up to 14 layers.

Additionally, we have extended our investigation to evaluate the potential of our developed SPRE sensing platform in detecting biomolecules. The biosensing experiment was performed by monitoring the real-time interaction of 1µM bovine serum albumin (BSA) and 100 nM Anti-BSA prepared in buffer solution (1X PBS, pH 7.4). The real-time response of the BSA-Anti B.S.A. interaction was recorded in SE using in-situ mode. The pre-functionalized Au sensor chip was mounted on the SPR cell and incorporated with the flow cell channel to perform the experiment. Before immobilizing BSA onto the Au surface, a stable baseline was recorded by passing PBS through the flow cell using a syringe pump with a flow rate of 5µl/min. Thereafter, 1µM BSA was exposed to the sensor chip with a flow rate of 3.335µl/min for 90 minutes. Afterwards, the BSA, PBS was used to rinse the sensor surface to remove the unbound BSA molecules. After 1 h washing with PBS, 100 nM of Anti-BSA was introduced to the sensor's surface with a flow rate of 5 µl/min for 1 h.

V. RESULTS AND DISCUSSIONS

The thickness of deposited Cr_4nm/Au_42nm layers over the glass substrate was characterized by ellipsometry by fitting the thickness. An optical model (in Complete Ease) is built after measuring the thickness with ellipsometry at 65°angle, as depicted in Fig.4a. The thicknesses of the Cr and Au layers were obtained around 3.87nm and 38.46 mm, respectively, with 2.12 nm of surface roughness. The lower MSE (mean square error) value of 3.92 (MSE <5) signifies the accurate fitting of the measured thickness. Fig. 4(b,c) shows the spectroscopic responses in terms of Ψ and Δ collected over a spectral range of 390-980 nm when the Au sensor chip is exposed to air and water media. A sudden drop in the Ellipsometry signal was observed at a wavelength of 600 nm when the analyte media was changed from air to water. These results indicate that the phase-matching condition is needed for the excitation of SPR, which was satisfied at different wavelengths, as reflected in the shifting of SPR dip position with the changing sensing analyte media (glycerol-water concentrations). As expected, a sudden shift in the phase signal can be seen in Fig.4(e,f), which occurs around the SPR position of the Ψ spectra, as shown in Fig.4(b,c). The sharp drop in the Δ signal around the SPR resonance position is due to the phase change after introducing the water and glycerol-water solution. The Δ signal is processed in the 0°<Δ<360° range by converting it into a positive value, as depicted in Fig.4f. The real-time response, as depicted in Fig.5(a-c), shows the maximum sensitivity in Ψ and Δ signal at the wavelength of 600 nm and 647 nm, respectively. It is observed that with the increase of RI, the Ψ signal increases stepwise while the Δ signal shows the opposite nature by decreasing step-wise. At the final step, after passing all glycerol concentrations, the sensor chip was again exposed to DI water to validate that the solution had no interfering effect as the signal returned to its initial position. The bulk RI sensitivity was obtained as 3106±171 nm/RIU, 815±29.6 °/RIU, and
5842±288 °/RIU with good linearity for wavelength modulation and amplitude and phase modulations as Ψ and Δ sensitivity, respectively, by the linear fitting of wavelength shifting, Ψ or Δ shift at a particular wavelength with respect to the RI change, as illustrated in Fig.5(d-f). The RI of the analyte solutions depends on the analyte concentration, directly influencing the shifting in ellipsometry parameters Ψ and Δ. Compared to the conventional SE phase signal, the shift in the SPRE phase signal is much higher. The RI variations for glycerol-water solutions have a dynamic range of 0.03 with good linearity, sufficient to detect protein analytes on the sensor surface. The RI sensitivity using our developed SPRE system is also compared in Table I with the other optical sensing techniques reported earlier.

Fig. 4. (a) Thickness fitting of Cr_4nm/Au_42nm coated on Si substrate. Spectroscopic response of bulk refractive index sensitivity in terms of (b,c) Ψ and Δ while Au sensor chip was exposed to air and water. (d,e) Ψ and Δ response while 0-25% concentrations of glycerol-water solutions were exposed to the sensor chip. (f) The plot for Δ a signal after converting it into a positive value.

The real-time in-situ responses for the layer-by-layer interaction of cationic PDDA and anionic PSS electrolytes in different time intervals are illustrated in Fig.6(a,b) at the wavelengths of 627 nm and 643 nm, which corresponds to the maximum shift in Ψ and Δ signals respectively. The Δ signal is more pronounced than the Ψ signal, and the SPR mode appears as a dip in the Ψ spectra where a sudden jump is observed in the respective Δ response, as shown in Fig.6(c,d).

Fig. 5. Real-time response of bulk refractive index sensitivity in terms of (a) Ψ (at 600 nm) and Δ (at 647 nm) while Au sensor chip was exposed to 0-25% concentration of glycerol-water solutions. The shift in Δ signal at different wavelengths when plotted (b) Δ variation from -180° to +180° and Δ variation from 0 to 360°. The bulk sensitivity plots for (d) wavelength shift, (e) shift in Ψ, and (f) Δ signal at wavelengths of 600 nm and 647 nm, respectively, with the refractive index variations.

Fig. 6. The real-time response of SPRE sensor for polyelectrolyte interaction. (a) Ψ signal at wavelengths of 627nm and 637nm (SPR mode). (b) Δ signal at wavelengths of 637nm (SPR mode) and 643nm during the interaction between PDDA and PSS electrolytes. The spectroscopic responses of (c) Ψ and (d) Δ signal for the PDDA and PSS layer-by-layer interaction on the sensor chip.
The shift in $\Psi/\Delta$ is significant at the wavelengths of 637 nm, which corresponds to the SPR resonance position, but the shift is lesser as compared to the responses at 627 nm and 643 nm for $\Psi$ and $\Delta$, respectively. The SPR mode in the $\Psi$ spectra shows a red shift with a step-by-step increase as the number of polyelectrolyte layers increases on the Au sensor's surface. Similarly, in $\Delta$ spectra, a red shift and a step-by-step decrement are observed with the increment of the number of polyelectrolyte layers deposited on the Au surface. The experimental results obtained serve as evidence that the SPRE sensing platform has the ability to successfully detect chemical species in a label-free manner.

![Fig. 7](image-url)

**Fig. 7.** (a,b) In-situ ellipsometry spectroscopic response of $\Psi$ and $\Delta$ for monitoring the interaction between 1 µM BSA and 100 nM Anti-BSA. (c,d) The real-time responses of $\Psi$ and $\Delta$ at the wavelength of 621 nm with the maximum sensitivity for the BSA/Anti-BSA interaction. Fitting of sensogram with measured (e) $\Psi$ and (f) $\Delta$ spectra at a wavelength of 621 nm.

The spectroscopic responses of (c) $\Psi$ and (d) $\Delta$ signal for the PDDA and PSS layer-by-layer interaction on Au sensor chip. The in-situ response of the SPRE sensor for monitoring the interaction between 1 µM BSA and 100 nM Anti-BSA was recorded in different wavelengths, and the maximum shifting in $\Psi$ and $\Delta$ spectra were observed at the wavelength of 621 nm, as illustrated in Fig.7. The spectroscopic responses for the BSA/Anti-BSA interaction are shown in Fig.7(a,b). After immobilizing 1 µM BSA, the real-time $\Delta$ signal shows a shift of 19.7° while a shift of 2.3° is observed in $\Psi$ signal at 621 nm wavelength of maximum sensitivity, as depicted in Fig.7(c,d). It is observed that the $\Delta$ response is more enhanced and 8.5 times higher than $\Psi$ response. Furthermore, introducing 100 nM Anti-BSA over this pre-immobilized BSA surface, the $\Psi$ and $\Delta$ signals show a 4° and 18° change, respectively. We obtained the association rate constants around $7 \times 10^5$ M$^{-1}$s$^{-1}$ and $11 \times 10^5$ M$^{-1}$s$^{-1}$ by fitting the sensogram plot for $\Psi$ and $\Delta$ respectively, as presented in Fig.7(e,f).

**TABLE I**

<table>
<thead>
<tr>
<th>Detection Technique</th>
<th>Sensor Chip Composition</th>
<th>Analyte</th>
<th>RI Sensitivity</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPRE</td>
<td>Au/Cr/Glass</td>
<td>Glycerol water</td>
<td>3106 nm/RIU ($\Psi$) &amp; 5842°/RIU ($\Delta$)</td>
<td>Our work</td>
</tr>
<tr>
<td>SPR</td>
<td>MOS2/Al</td>
<td>water</td>
<td>483.2-973.7 nm/RIU.</td>
<td>[40]</td>
</tr>
<tr>
<td>Tapered Optical Fiber-based SPR</td>
<td>Au film</td>
<td>Aqueous NaCl</td>
<td>1395-1914 nm/RIU.</td>
<td>[41]</td>
</tr>
<tr>
<td>SPR</td>
<td>Au-coated nanograting structured laminating plastic</td>
<td>Glycerol-water</td>
<td>800 nm/RIU.</td>
<td>[42]</td>
</tr>
<tr>
<td>WHispering Gallery Mode (WGM.) Optical Interferometry</td>
<td>Si/Graphene (6 layers) micro-disk resonator microstructure d Optical fiber</td>
<td>Aqueous NaCl</td>
<td>355.5 nm/RIU. (first mode)</td>
<td>[43]</td>
</tr>
<tr>
<td>SPR</td>
<td>ZnO/Au/Graphene</td>
<td>Liquid</td>
<td>66°/RIU</td>
<td>[44]</td>
</tr>
<tr>
<td>Hybrid Photonic Crystal-Plasmonic</td>
<td>Ring resonator/Si Photonic Crystal</td>
<td>Gascous</td>
<td>1250 nm/RIU.</td>
<td>[45]</td>
</tr>
<tr>
<td>LSPR</td>
<td>Au nano cross-array/SiO$_2$/Au/ SiO$_2$</td>
<td>Liquid</td>
<td>880 nm/RIU.</td>
<td>[46]</td>
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<tr>
<td>SPR</td>
<td>Au/2D materials hybrid structure</td>
<td>-</td>
<td>15°/RIU</td>
<td>[47]</td>
</tr>
<tr>
<td>SPR</td>
<td>Au[(GO)x(G Oy)]</td>
<td>Glycerol-water</td>
<td>150°/RIU</td>
<td>[48]</td>
</tr>
</tbody>
</table>
For a 0.001° phase measurement accuracy, RI resolution was estimated as $1.712 \times 10^{-7}$ RIU. The LOD was found to be around 5.2 pM for Anti-BSA. The sensorgram was fitted by using a Pseudo first order differential equation (equation 9) to estimate the binding kinetics parameters (i.e., association ($k_a$) and dissociation rate constant ($k_d$) etc.) [50]–[52],

$$\frac{dR}{dt} = k_a[C](R_{\text{max}}R) - k_dR \tag{9}$$

### TABLE II

<table>
<thead>
<tr>
<th>Detection Technique</th>
<th>Sensor Composition</th>
<th>Detected Species</th>
<th>Sensitivity or LOD</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPRE</td>
<td>Glass/Cr/Au</td>
<td>Anti-BSA</td>
<td>5.2 pM</td>
<td>Our work</td>
</tr>
<tr>
<td>SPR</td>
<td>Au/[GO(+)/G</td>
<td>Anti-BSA</td>
<td>6 µg/ml</td>
<td>[49]</td>
</tr>
<tr>
<td>Fiber optic SPR</td>
<td>Core(polymer)-shell (Au) Nanodomes with nanogaps</td>
<td>Thyroglobulin</td>
<td>38 fg/ml</td>
<td>[53]</td>
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<tr>
<td>SPR</td>
<td>Ag/Silica-coated Ag nanohole array</td>
<td>Biotinylated diphosphatidylcholine</td>
<td>450 nm/RIU</td>
<td>[54]</td>
</tr>
<tr>
<td>SPR</td>
<td>Streptavidin-coated Au film</td>
<td>Neuregulin 1</td>
<td>200 pM</td>
<td>[55]</td>
</tr>
<tr>
<td>Electrochemical</td>
<td>Graphene-hydrated biopolymer</td>
<td>Fumarate</td>
<td>0.60 nM</td>
<td>[56]</td>
</tr>
<tr>
<td>Electrochemical</td>
<td>ITO-Ti/Au/Au-nano dendrites</td>
<td>Prostate cancer biomarker</td>
<td>0.80 nM</td>
<td>[57]</td>
</tr>
<tr>
<td>Electrochemical</td>
<td>G.C.E./Chitosan-CD/D.N.A.</td>
<td>Nitrosodimethylaniline</td>
<td>9.9 nM</td>
<td>[58]</td>
</tr>
<tr>
<td>Electrochemical-LSPR</td>
<td>Au-Ag nanocone</td>
<td>Sialic acid</td>
<td>17 µM</td>
<td>[59]</td>
</tr>
</tbody>
</table>

The experimental result of the detection limit of Anti-BSA from our developed SPRE system is compared with the other biosensors reported earlier, as listed in Table II. The comparison shows that our integrated sensing technique is highly sensitive, has better resolution and can be recognized as a label-free optical biosensing platform by utilizing the custom-designed, in-house-built 3D-printed set-up. Additionally, SPRE can be used to monitor interior surfaces, enabling the detection of metal surface adsorption.

### VI. CONCLUSION

This work summarizes the development of the SPRE instrumentation, where advantages of both SPR and Ellipsometry techniques can be utilized by avoiding the limitations of individual methods. The 3D-printing technology has been used to build appropriate components with customized designs relevant to our particular requirements at a low cost and in less time. We have constructed a compact, user-friendly, lightweight, durable, easy-to-clean, and cost-effective 3D-printed SPR module that is designed to seamlessly integrate with a commercial ellipsometer. Our developed SPR module has a simpler configuration similar to the SPR Kretschmann configuration that eliminates the difficulty in optical alignment, which is a major issue while integrating with the ellipsometer. This SPRE set-up could also be employed for individual SPR sensing and is preferable over expensive commercial SPR systems. This SPR module has the advantage of integrating with other powerful sensing tools, such as electrochemical sensing. The experimental findings using our proposed SPRE sensing platform demonstrate its ability to perform label-free, efficient, and real-time optical sensing for chemical and biological species. Furthermore, the theoretical modelling in ellipsometry is also able to provide qualitative and quantitative information about the target analytes.

The bulk RI sensitivity was obtained around $3106\pm 171$ nm/RIU, $835\pm 29.5$ °/RIU, and $5842\pm 288$ °/RIU for wavelength interrogation, $\Psi$ and $\Delta$ respectively, using the different concentrations of glycerol-water solutions. The real-time in-situ response for the interaction of PDDA (cationic) and PSS (anionic) electrolytes (up to 14 layers) was also monitored to mimic the label-free sensing ability of our developed SPRE platform. Also, this SPRE system was utilized to establish a label-free, highly sensitive, efficient biosensing platform by monitoring the standard BSA/Anti-BSA protein interactions. We achieved LOD around 5.2 pM for the sensitive detection of Anti-BSA analyte using BSA as a ligand with an association rate constant of $11\times 10^5$ M$^{-1}$s$^{-1}$ (for $\Delta$ response). The experimental results using our developed SPRE platform were compared (Table I,II) with other reported works to compare the sensitivity and efficiency. SPRE is a highly promising optical technique for bio-chemical sensing and biomedical applications. The utilization of the 3D-printing technology enables the production of such devices, which in turn facilitates the development of an integrated sensing platform that is highly surface-sensitive and can be employed for different sensing purposes. Since an ellipsometer is comparatively costly,
the single wavelength ellipsometer can also be used to reduce the cost. To the best of our knowledge, there is no earlier report of manufacturing such a compact and cost-effective 3D-printed SPR module that has been explicitly designed for efficient SPRE sensing. Though the ellipsometer is costly, the integration with SPR provides enhanced sensitivity, measurement accuracy, precise analysis, and more information. We hold a positive outlook on the potential of our proposed SPR module that would undoubtedly attract researchers from diverse backgrounds and motivate them to explore the use of the SPRE platform in different sensing applications efficiently at a low cost. We also anticipate that our present work will inspire researchers to grasp the efficacy of 3D printers for accomplishing in-house-built customized appliances without the help of external sources or manufacturers at low cost and in less time.

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REFERENCES


[18] G. Hu et al., “High-precision measurement of optical constants of ultra-thin coating using surface plasmon...


