Advanced Plasmonic Biosensing Devices and Automation Systems for Disease Diagnostic and Drug Screening Applications

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Abstract

Bio-plasmonics is proposed for the research and development of novel devices, which use biomolecules as a part of the plasmon oscillation system to actively interact with nano/micro structure. We have reported a novel design of Surface Plasmon Resonance (SPR) device and system, which uses alternative dielectric layers to enhance the SPR signal quality and modulate its resonant position. The use of biomolecular thin film, such as DNAs or Proteins, in this design can result in resonant condition of wavelength changes and thus can be detected by using nano-grating in the scattering mode with enhanced feature due to resonance. According to our calculation, it can provide ultra sensitivity system (dLx/dn) of 10\(^{-4}\) for biosensor applications. The fundamental SPR principle and extended application of these fundamental principles and novel devices, including screening and diagnosis, will be discussed SPR-system to control is the ability. SPR-system has the ability to detect, in near real-time, the concentration of a target analyte and biosensing is viewed as a key application domain for this new technology.

Keywords: Bio-plasmonics, surface plasmon polaritons (SPPs), DNAs, Proteins, biosensor.

1. INTRODUCTION

Advancements in biotechnology have culminated in its integration with semiconductor technologies such as micro-electromechanical systems, resulting in the evolution of biosensors. Biosensors provide applications throughout the process tools for speedy development of drugs and accurate diagnosis and understanding of biological mechanisms [1]. The most widespread label-free detection systems found on the market are based on surface-plasmon resonance (SPR) [2, 3]. Biochemical interactions at the sensor surface are monitored by observing the resonant behavior of guided waves at a thin metal film. Today, the market leader is the company BIACORE [4]. Other companies include Applied Biosystems [5] and Texas Instruments [6]. Currently, we are developing an integrated SPR device, which couples a hand-held SPR system, we have also developed three kits of micro-prism coupling [7], nano-grating [8] and active-plasmonic [9-11] sensors for further minimization of the whole system. We also propose to use dip pen nanolithography to create ordered array of nano size spots to study global dynamic response of molecules based on average of stochastic behavior. The long-term objective is to promote the quality and quantity of novel medical devices and thus better health care through operable medical standards and regulations.

2. THEORETICAL INSIGHTS

In the past decade, the phenomena of surface plasmon resonance (SPR) have been extensively used to investigate optical constants and thickness of thin films, surface properties [12], and molecular interactions on the solid–liquid interface [13-17]. The SPR is a charge-density oscillation that may exist at the interface of two media with dielectric constants of opposite signs, for instance, a metal and a dielectric. This phenomenon was first observed in metal grating in the early 1900s. Kretschmann used a metallic-film-coated (~50 nm) prism to generate a surface plasmon resonance (SPR) signal [15]. Since then, the Kretschmann prism-coupling device has been used extensively to study the optical properties of metallic thin films, including index of refraction (n), extinction coefficient (k), thickness (d), and roughness [18]. For the practical applications of SPR sensing device, we have to apply Frensel equation to calculate the total reflectance from a grating structure. Such a periodic metal nanostructures can have very interesting and exciting optical properties which strongly depend on the used materials, layer thickness, and grating pitch. Other than the traditional Kretschmann (attenuated total reflectance (ATR method)) and Otto (frustrated total reflectance (FTR)) configurations, several novel devices based on grating nanostructure of mixed hybrid configuration have been reported in literatures, which include long-range surface plasmon resonance (LRSPR) [19-21] and coupled plasmon waveguide resonance (CPWR) [22].

The nature of plasmon resonance comes from the light-matter interactions and the behavior of free electrons in the metal can be well described by harmonic oscillator model with external applied force or EM field. Surface plasmon wave can interact with photon if the momentum or energy is matched under certain conditions. But incident light cannot excite SP wave directly from the dielectric medium. For a given frequency, \(\omega\), light traveling in the dielectric will have a wave vector (\(k\)).
\[
\begin{align*}
\frac{k}{c} \cdot \sqrt{\varepsilon_r} = \frac{1}{k}.
\end{align*}
\]  
(1)

The parallel component of this wave vector in the plane of the incident surface,
\[
\begin{align*}
k_x = \left( \frac{\omega}{c} \right) \sqrt{\varepsilon_r} \sin \theta.
\end{align*}
\]  
(2)

Because the propagation constant of SP wave is always higher than that of wave propagation in the dielectric, SP wave cannot be excited directly. This momentum change can be achieved by using: 1. micro-prism coupling, 2. diffraction gratings, and 3. active-SPR devices. It was found that the prism coupling method can have highest resolution \((5 \times 10^{-7} \text{ refractive index units (RIU))}.\) With a coupling prism, the in-plane component of the propagation constant can have a cross point with the SP dispersion relation curve. The \(k_x\) varies with the incident angle \((\theta)\). If \(\omega_L\) is the frequency of incident light then its momentum will match the \((k_{sp})\) SP propagation constant at the metal/air interface.

\[
\begin{align*}
k_x = k_{sp} \Rightarrow \sqrt{\varepsilon_r} \sin \theta = \frac{\varepsilon_r - \varepsilon_m}{\varepsilon_r + \varepsilon_m}.
\end{align*}
\]  
(3)

With negative dielectric constant of metal \((\varepsilon_m)\) and positive dielectric constant of material \((\varepsilon_r)\), the discontinuous \(E_z\) electric field will have two opposite propagating direction at the interface. It results in the surface charge density oscillation and the macroscopic oscillation of electric field in the two media with components along the x and z axis directions. This occurs at a different frequency to the bulk oscillations and is confined to the surface and vanished to both sides of the metal surface. Since the surface density alternates in sign, the spatial summation in the z axis direction gives an exponential decay in the magnitude of the electric field. The surface plasmons wave vector \(k_x\) is a complex function; \(k_x = k_x' + ik_x''\), hence the surface plasmons propagate in the x direction and decay in amplitude as \(exp(-2k_x' x)\). Thus we define a propagation length \(L_x\),

\[
L_x = \left(2k_x'\right)^{\frac{-1}{2}}.
\]  
(4)

Because surface plasmon \(k_x > k_0\), surface plasmon wave amplitude decay in z-direction as \(exp(-k_{z,2}|z|)\). We can define the skin depth of surface plasmon wave propagation \(Z\) axial,

\[
Z = \frac{1}{|k_{z,2}|}.
\]  
(5)

In Figure 1, the exponential decay of electrical field on the Au/air interface has both z and x directions with different space constants in nm and um scale. It can be shown that this SPR wave on the surface subjected to the effects of wavelength, incident angle, metal and dielectric medium. It thus opens up a wide spectrum of possible engineering designs for SPR devices – i.e. plasmonics [23].

3. MATERIALS AND METHODS

It is well known that SPR can be excited by three different coupling mechanisms, i.e., micro-prism, grating, and active-SPR couplings as shown in Fig. 2. It is now better understood that we can use engineering design for the fine tuning of the coupling conditions of different applications.

![Fig. 1. Illustrations of the origin of free electrons oscillation in an applied EM field and the excitation of SP wave on the metallic surface with nm scale in z axis and um scale in x axis.](image1.png)

![Fig. 2: We have also developed three kits of (a) micro-prism coupling, (b) nano-grating and (c) active-plasmonic sensors for further minimization of the whole system.](image2.png)

A. Micro-prism coupler:

For example, the refractive index of coupling prism is a critical factor for the design of SPR sensor to couple photons to plasmons or vice versa. At 633 nm of incident light, the higher refractive index, from PDMS \((n=1.4)\) to Glass substrate \((n=1.5)\), will cause smaller SPR angle. This is equivalent to use longer wavelength in the visible-near infrared region, which will allow penetration of silicone substrate for using single crystal Si wafer as a coupling prism [24]. This would normally result in 2-5 times sensitivity enhancement and better surface preparation due to smoothness.

The experimental process flow is depicted in Ref.7. The square-based photoresist plates are made by...
photolithography processes, and then these plates are treated with thermal reflowing process to transform these plates into the shape of micro-prism arrays (MPAs) on the substrate. Subsequently, the cylinder shape will turn into hemisphere, just like plano-convex lens array. And then, PDMS mold is formed after thermal curing, the MPAs film is done after UV forming techniques. The replicas of micro-prism arrays on PDMS films are made. Finally, PMMA films with or without micro-prism array are attached on the planar glass substrate using refractive-index matched oil. The RI model: \( D=10 \mu m, G=2.9 \) and \( 12.3 \mu m \).

For our experiments, we prepared one-dimensional patterns of nanostructures by electron beam lithography ELS-7800EX (ELIONIX CO.), the line size is 200nm, the pitch 400nm and 100nm thick resist ZEP520A on silicon substrates [8]. The shape of the individual grating was slightly elongated, exposure area 1.2×1.2 \( mm^2 \). In model experiments, a minute change in ambient refraction index resulted in the observable shift of the angle and intensity change in the reflectance as shown in Fig. 2(b). This means that angle shift detection in SPR schemes offers higher sensitivity and miniature system by several orders than the traditional intensity measurements. The purpose of this article is to survey the latest results. They embrace an exhaustive understanding of SPR properties and applications of miniature plasmonic to create (bio)chemical sensors with ultra-high sensitivity and wide dynamic range. For grating coupling, it has been well known that one can control the radiation field (angle and intensity) by controlling the pitches of grating and incident angle. However, it experiences enhanced localized EM field when the scale approaching nanometers. Recent efforts in enhanced transmission of metallic nano holes array have triggered a lot of activities in this surface plasmon related phenomena. It has demonstrated that this 1D or 2D grating patterns will create a photon forbidden zone and thus has been named as photonic band-gap devices [8].

### B. Nano-grating coupler:

For our experiments, we prepared one-dimensional patterns of nanostructures by electron beam lithography (EBL). We fabricated two devices, 2-layer (Alq\(_3\)/Au) and 4-layer symmetrical organic dielectric films (Alq\(_3\)/Au/Alq\(_3\)/Au), with grating line width and pitch size of 400 nm and 800 nm, respectively. We used the emission of Alq\(_3\), organic molecules to excite SPPs on multilayer grating coupled emission as shown in Fig. 2(c). The emissions correspond to the resonant condition of SP modes on the Alq\(_3\)/Au interface and grating couple to the Au/air interface for the emission of light. This technique has surface plasmon grating coupled emission (SPGCE) of light passing through metal and is a multilayer grating approach for the excitation of SPPs. Our experimental results show that these devices can have specific directional emission, enhanced emission intensity, and reduced Full-Width Half-Maximum (FWHM). Further investigations will facilitate the development of novel bio-sensing device having multilayer organic/metal nanostructure for grating coupler active plasmonic biosensor [9-11].

#### D. Coupler SPR experimental set up:

The SPR measurement system set-up used for the described study was based on a grating coupled configuration, which is also an extremely versatile new measuring instrument. The schematic description of the setup is depicted in Fig. 4.

We have set up a SPR photoluminescence (PL) and electroluminescent (EL) measurement systems (Fig. 4) for the angular emission spectra produced by the designated surface plasmon grating coupled emission (SPGCE) from nanostructure. In brief, a 405 nm light source (Spectral Luminator 69050, Newport Oriel Inc., USA) or 405 nm diode laser (BWB-405-20E (20mW), B&W TEK Inc, USA) is used to excite Alq\(_3\) molecules on the nano-grating device. The device was placed at the center of a high-resolution rotary stage with computer controllable incident angle, \( \theta \), emission angle, \( \theta_e \), and azimuthal angle, \( \phi \). The SPGCE output light was collected and measured by a 2-inch lens with focal distance of 5 cm and a 12-bit spectrometer (USB2000, Ocean Optics Co., USA). Two motorized rotary stages (SGSP-120YAW-W, Sigma Koki, Japan) and its controller (SHOT-204MS, Sigma Koki, Japan) are used to control \( \theta \) and \( \theta_e \), between sample and detector stages. The nominal angular resolution is 0.0025 degree. The system uses temperature, humidity sensor (Galiltec TFG80J) and data acquisition card (PCI-6070E, National Instrument Inc, USA) to monitor the environmental operating condition for higher accuracy and reproducibility.
E. System operation modes:

There are six basic modes of operation that were used to perform measurements with the previously described set-up:

1. Sample stage (0)/detector stage (0) mode: this is the so-called tuning-mode. It was used to find the most efficient excitation condition. The grating was rotated and moved through the resonance while the spectrometer rotated by the same angle to maintain the same sample perspective.

2. Sample stage (0)/detector stage (20) mode: the 0/20-mode was used to take measurements of reflectivity versus angle of incidence for grating coupled SPR images. If the sample is rotated to a given angle of incidence 0, the detector arm has to move by 20 in order to collect the reflected beam with the CCD images for GWC system.

3. Sample stage (Fixed)/detector stage (Fixed) mode: (detector stage scan): angular fluorescence spectra were measured in this mode. The grating was kept at a fixed angle of incidence to excite organic semiconductors by grating coupled surface plasmons emission. The spectrometer moved to measure the fluorescence intensity at different emission angles relative to the surface normal of the grating.

4. Sample stage (0)/detector stage (Fixed) mode: (sample stage scan): angular fluorescence spectra were measured in this mode. The grating was kept at a scan angle of incidence to excite organic by grating coupled surface plasmons emission. The spectrometer moved to measure the fluorescence intensity at different emission angles relative to the surface normal of the grating.

5. Azimuthal-angle-dependent mode (azimuthal-angle scan, local rotary 0–360°): This set-up mode was used to record azimuthal-angle-dependent reflectivity data from a diffraction grating. The grating was placed on the rotating table with the incident light at a fixed stage and detector angle to measure the fluorescence intensity.

6. Kinetic-mode: in kinetic-mode the spectrometer was at rest with the grating fixed at a convenient excitation angle. This mode was used to study the binding of substances to the sensor surface as a function of time. The nature of the experiment determined which detector was the appropriate one. Hence there were two ways of measuring kinetics. For studies based on reflectivity, the CCD was fixed at an angle equal to twice of the chosen excitation angle.

4. Results and Discussion

A. Micro-prism coupler:

The Fig. 6 shows the top-view images of fabricated through an optical microscope MPAs microscope (Olympus BX 60) with Polaroid digital microscope camera (DMC) of 100x digital images patterned.

Three different geometrical configurations (RI, SR and SH) and their effect on the MPAs coupling SPR responses are shown in figures 7, respectively. Different MPAs configuration result in a different reflectivity curves. Coupling ratio of (D=10 μm, Gap=8.9 μm) appears to have highest coupling efficiency on 533nm and 633nm incidence light. The measured results of different coverage ratio and base area are summarized in Table 1. The MPAs with higher coverage ratio, smaller base area and higher height ratio exhibits higher coupler efficiency. The MPAs coupler SPR with coverage ratio, smaller base area and higher height ratio exhibits higher coupler efficiency.

<table>
<thead>
<tr>
<th>MLA</th>
<th>RI model: D=10 (μm)</th>
<th>SR model: D=10 (μm)</th>
<th>SH model: D=10 (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size (μm)</td>
<td>G=2.9</td>
<td>G=12.3</td>
<td>G=25</td>
</tr>
<tr>
<td>Coverage Ratio (CR%)</td>
<td>60%</td>
<td>20%</td>
<td>50%</td>
</tr>
</tbody>
</table>
Fig. 7. The MPAs coupler SPR to demonstrate the different wavelength of TM-polarized modes. (a) RI model: D=10 μm, Gap=12.3 μm (b) SR model D=10 μm, Gap=8.9 μm. (c) SH model: D=10 μm, Gap=11.3 μm.

B. Nano-grating coupler:

The sensing layer is for the detection of small quantities of a given substance, the grating surface has to be functionalized in order to specifically bind the target molecules to it. For each molecule to be analyzed, a specific counterpart is needed. Every molecule, for which a selective counterpart is found, may in principle be measured with the sensor system. An illustration of the recognition process on the chip surface is shown in Fig 8.

Fig. 8. Metallic grating surface on Bio-chemical adlayer sensing.

This allows us to measure the experimental angle-dependent reflectivity as shown in Fig. 9. Incident light beam of different wavelength 530nm, 643nm and 833nm on a grating of this pitch permits momentum enhancement such that two SPR angles appear. These are the first and second order resonances, where the number and sign refer to the diffracted order that provides the resonant coupling to the SPP.

Fig. 9. Illustrate the azimuthal-angle-dependent scans at θ=45 degree. Two minima in reflectivity can be observed which are attributed to the coupling to a surface plasmon in first and second order.

The resonance peaks for the in coupling grating were rotary table measuring angle φ=0 degree, the intensity of the reflected light beam is recorded on the different angle (θ=5–25 degree, φ=0 degree) of observation. The SPR signal was measured as a change in the angular distance between the SPR dips in the angular spectrum as shown in Fig. 10(a). Summary of experimental results for metal grating sensor, compared with the sensitivity of a light 643 nm and 833 nm gauge repeatability as shown in Fig. 10 and Table 2.

Fig. 10. The measured of the reflected light angle from the metal grating for TM-polarised light with a wavelength of (a) 530 nm, (b) 643 nm, and (c) 833 nm.

C. Active-plasmonic coupler:

We have measured SPGCE spectra for each of our fabricated samples, as shown in Fig. 11, by using PL measurement system to collect the emitted light intensity from -10 to 15 degree with 1 degree per step. We can then also examine the effect of SPPs excited SPGCE on the angular dispersion of emission spectra. The resultant angular emission spectra of enhanced luminescence from metal/organic grating from multiple emission angles are composed into color coded three dimensional spectrogram as shown in Figs. 12(a) and 12(b) for 2-layer and 4-layer device, respectively. It is quite obvious from these two figures that 4-layer one does have higher intensity and smaller FWHM. The average shift in peak wavelength is 14 nm/degree for the grating with pitch size of 800 nm. The emission spectra can shift from 750 nm to 480 nm by changing the emission angle for measurement. It results in an angular dependent tunable color device with specific structural parameters, e.g., pitch constant (Λ), the thickness of each layer of the grating and the optical indices of used materials, to satisfy the SPP resonant conditions.

Table 2. Summary of grating pitch 400 nm for SPR resonance angles

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>Angle (Degree)</th>
<th>Air</th>
<th>Water</th>
<th>NaCl</th>
<th>NaCl</th>
<th>Chloroform</th>
<th>Chloroform</th>
<th>Alcohol</th>
<th>Alcohol</th>
</tr>
</thead>
<tbody>
<tr>
<td>530 nm</td>
<td>12.8</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>833 nm</td>
<td>64.4</td>
<td>64.26</td>
<td>64.25</td>
<td>64.3</td>
<td>64.16</td>
<td>63.06</td>
<td>62.90</td>
<td>62.8</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 11. SEM images of the gratings cross section, which show the arrangement of a periodically lamellar layer a) 2-layer structure of Alq3/Au and b) 4-layer structure of (Alq3/Au)/Alq3/Au on top of a 100 nm PR structure.
The enhanced emission spectra measured with the coupling at different angles, as shown in characteristic diagram of PL emission in polar coordinates (Fig. 12(c)) for planar, 2-layer, and 4-layer, respectively. The ratio of the maximal intensity of these three devices is 1:4:6. The intensity from the 4-layer structure can be strongly enhanced by recovering from three possible mechanisms, i.e., coupled SPP from Alq3 scattering emission, non-radiative mode, and the long-range plasmon polaritons (LR-SPPs) with symmetrical dielectric structure. The LR-SPPs are associated with the interactions of symmetric or antisymmetric magnetic fields on both sides of metal interface [23, 25]. The fields can constructively interact inside the thin metal film (20 nm) and then result in the LR-SPPs, which can extend into both Alq3 layer for excitation.

**Fig. 12.** The PL emission obtained from a grating sample having 2-layer and 4-layer structure (grating size: line 400 nm, pitch 800 nm, area size 1.2×1.2 mm2). The 4(a) and 4(b) are shows PL 3-D emission image obtained from a grating sample. The dependence of the emission spectra on observation angle (0°) is shown in 4(a) and 4(b) for 2-layer and 4-layer structure, respectively. The 4(c) shows the planar, 2-layer and 4-layer. The emission maximum was about 0° and -3° for 2-layer, 4-layer devices, respectively.

**V. CONCLUSIONS**

In this study, we investigated the micro-prison and nanostructures metallic grating with active-SPR device by using electron beam lithography of SPR on optical Biosensor. We have shown experimentally that strong coupled between electronic and photonic resonances in plasmonic bio-sensing device. In experiments we showed that SP-coupled also occurs with grating gold films, we will making this technology applicable to any assay using metal grating films such as SPR to measure bioaffinity reactions. For the future, Bio-plasmonics plans to develop products and applications for bio-safety tests, clinical diagnostics, drug screening, and for drug discovery and biomedical research.

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