Single-beam self-referenced phase-sensitive surface plasmon resonance sensor with high detection resolution

Shu-Yuen Wu (胡樹源) and Ho-Pui Ho (何浩培)

Department of Electronic Engineering, The Chinese University of Hong Kong, Hong Kong

Received August 30, 2007

A versatile and low-cost single-beam self-referenced phase-sensitive surface plasmon resonance (SPR) sensing system with ultra-high resolution performance is presented. The system exhibits a root-mean-square phase fluctuation of $\pm 0.0028^\circ$ over a period of 45 min, i.e. a resolution of $\pm 5.2 \times 10^{-9}$ refractive index units. The enhanced performance has been achieved through the incorporation of three design elements: a true single-beam configuration enabling complete self-referencing so that only the phase change associated with SPR gets detected, a differential measurement scheme to eliminate spurious signals not related to the sensor response, and the elimination of retardation drifts by incorporating temperature stabilization in the liquid crystal phase modulator. Our design should bring the detection sensitivity of non-labeling SPR biosensing closer to that achievable by conventional fluorescence-based techniques.

OCIS codes: 240.6680, 310.6860.

Over the past two decades, optical sensors based on the surface plasmon resonance (SPR) phenomenon have gradually evolved to become a standard tool for quantitative measurement of chemical and biological binding reactions[1]. In order to fulfill the ever-increasing demand of application requirements, SPR sensing instrumentation has been improving continuously in terms of resolution, system stability, measurement throughput and cost. One approach for achieving ultra-high resolution is to measure the SPR phase response. The SPR phase undergoes a steep jump across the resonance dip, which means that a small refractive index change near the sensing surface may lead to a large signal response. SPR phase detection was first demonstrated through heterodyne interferometry[2]. This sensor gave a resolution of 5×10^{-7} refractive index units (RIU), which was already quite a significant improvement at the time. Later, the Zeeman laser has been employed to reduce system complexity[3]. The beat frequency (typically in kilohertz to megahertz range) caused by interference between the two orthogonal polarizations within the Zeeman laser beam provided a direct measurement of the SPR phase through measuring the phase difference in the optical beam immediately before and after traversing the sensor head. The main drawback of this approach is its difficulty in operating at low frequencies required by imaging measurement — an important attribute for achieving two-dimensional (2D) SPR sensor arrays with high measurement throughput. In order to address this issue, it has been reported that a liquid crystal modulator (LCM), which may be used as a voltage-controlled optical retarder, can be incorporated to perform SPR phase measurement through a five-step phase-shifting method[4]. Indeed, this approach achieved a measurement resolution of 2×10^{-7} RIU. It should be mentioned that LCM has also been used by Hooper and Sambles[5]. More recently, quadrature interferometry of SPR phase using a LCM has also been demonstrated by Lee *et al.*[6]. Their system achieved an experimental refractive index resolution of 6×10^{-6} RIU.

In spite of its simplicity as a retardation modulator, LCM has its own disadvantages, namely high temperature dependence, high nonlinearity, low operation frequency and limited retardation modulation range. Small variations in temperature will result in sizeable measurement drift. Indeed the situation will be much more problematic for the phase-shifting approach reported by Su *et al.*[4], as the accuracy of the final phase measurement relies entirely on the repeatability of the five data points obtained upon shifting of the retardation within a 2π circle. From the engineering point of view, LCM offers a number of advantages such as low cost, long lifetime, small size, no mechanical moving parts, low power consumption, light weight and low operation voltage. LCM is very suitable for portable SPR biosensor instruments if all of its shortcomings can be removed. With practicality and high measurement resolution as the objectives, we construct a single-beam self-referenced phase-sensitive SPR sensing system demonstrating three important features. Firstly, the single-beam construction significantly reduces complexity and completely removes the need of any beam alignment for obtaining high interference efficiency. Secondly, extremely high stability is achieved through the incorporation of temperature control to stabilize the retardation drift in the LCM, and the use of differential phase measurement eliminates unwanted phase errors caused by the ambient. Thirdly, a multi-pass beam-folding device is implemented to extend the retardation modulation depth into multiples of 2π, which provides better accuracy in the phase extraction process.

For the operation principle of our system, we need to mention that SPR only affects the p-polarization of the incoming light. The s-polarization remains unperturbed and may be used as the reference. With it being single-beam self-referenced (i.e., we are performing interference between the s- and p-polarization within the beam itself), our system has built-in capability for complete elimination of unwanted noise coming from the environment, as phase changes due to external disturbances other than

1671-7694/2008/030176-03

that coming from SPR should be present in both of the two orthogonal polarizations. The resonance condition of SPR is primarily influenced by the refractive index of the medium near the sensing surface. In our system, the phase difference between the s- and p-polarization (i.e. retardation) may come from three effects: 1) SPR, which is the quantity we aim to measure; 2) artificial retardation modulation introduced by the LCM; 3) unwanted phase polarization effects associated with the optical components. The fact that our system is inherently a self-referencing as well as differential one, it is likely that we are only left with the third item to deal with. From our experimental results, it is shown that errors due to this item are extremely small, hence leading to very high measurement resolution. When the incident light beam is set at angle $\theta_{\rm i}$ relative to the normal direction of the metallic surface in the SPR coupling prism, the complex reflection coefficients of p- and s-polarization can be expressed as

$$r_{\rm s,p} = \frac{r_{12} + r_{23}\exp(2ik_{z1}d)}{1 + r_{12}r_{23}\exp(2ik_{z1}d)}, \quad r_{ij} = \frac{Z_i - Z_j}{Z_i + Z_j},$$

for p-polarization, $Z_i = \varepsilon_i/k_{zi}$; for s-polarization, $Z_i = k_{zi}$. $k_{zi} = k_o(\varepsilon_i - \varepsilon_1 \sin^2\theta_{\rm i})^{1/2}$. Z is an impedance quantity, k_{zi} is the component of the wave vector in medium i in the normal direction of the metal thin film, k_o is the wave vector in vacuum and ε_i is the dielectric constant of medium i. Amplitude reflection coefficients $r_{\rm s,p}$ can be rewritten as

$$r_{\rm p} = |r_{\rm p}|\,{\rm e}^{i\phi_{\rm p}} \quad \text{and} \quad r_{\rm s} = |r_{\rm s}|\,{\rm e}^{i\phi_{\rm s}}.$$

The phase difference between p- and s-polarization is therefore $\phi_{\rm p} - \phi_{\rm s}$. The detected signal from the probe channel ($V_{\rm det}$) with the retardation caused by SPR and the LCM may be described as

$$V_{\rm det} \propto E_{\rm m}^2 = (E_{\rm os}\cos\theta_{\rm s})^2 + (|r|^2 E_{\rm op}\cos\theta_{\rm p})^2$$

$$+2(E_{\rm os}\cos\theta_{\rm s})(|r|^2 E_{\rm op}\cos\theta_{\rm p})\cos(\Delta\psi_{\rm LCM} + (\phi_{\rm p} - \phi_{\rm s})),$$

where $E_{\rm m}$ is the amplitude of the detected light; $E_{\rm op}$ and $E_{\rm os}$ are the amplitudes of the p- and s-polarization; r is the reflection coefficient of the SPR sensor head for the p-polarization; $\theta_{\rm p}$ and $\theta_{\rm s}$ are the off-axis angle of the polarizer between p- and s-polarization axis (45° for the present setup) respectively; $\Delta\psi_{\rm LCM}$ is the phase change introduced by the LCM.

$\Delta\psi_{\rm LCM}$ can be found by analyzing the reference signal traces upon varying the driving voltage of the LCM. At the same time if we perform the same procedures on the probe signal traces, then we should expect to find $[\Delta\psi_{\rm LCM} + (\phi_{\rm p} - \phi_{\rm s})]$, as the beam would have then been further modified by the SPR sensor head. This means that if we continuously analyze the signal traces from the reference channel as well as the probe channel, we should be able to find $(\phi_{\rm p} - \phi_{\rm s})$, the SPR phase change, through a phase subtraction step. Obviously the differential measurement approach will help us improve stability by minimizing the effects of unwanted changes in the characteristics of the LCM and also other disturbances from the surrounding environment.

Our experimental setup is depicted in Fig. 1. This

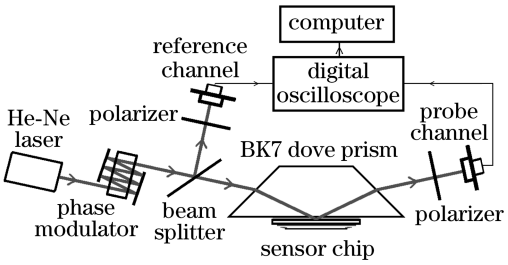

Fig. 1. Experimental setup of LCM-based SPR sensing system.

simple configuration is essentially a single-beam self-referenced differential phase-sensitive SPR sensor system capable of achieving high resolution. Large retardation modulation is obtained by placing the LCM element between two mirrors so that the optical beam travels through the modulator multiple times before leaving the cavity. The fast axis of the LCM is aligned to the p-polarization of the laser beam in order to obtain maximum retardation modulation depth. The LCM (Newport LCR-VIS-05) is driven by a controller (Newport 932-CX) which accepts an external drive signal for producing the required retardation modulation. In our case, we used a programmable function generator (Hameg HM8130) to generate a 10-Hz saw-tooth waveform oscillating between $0-13$ V for achieving maximum retardation modulation depth. The light source is a 12-mW linearly polarized He-Ne laser at the wavelength of 632.8 nm. The optical polarization axis of the laser beam is set at 45° from both p- and s-polarization orientation so that the optical power is initially divided into two equal halves along the p- and s-polarization axes. However, the power distribution may be adjusted as necessary for signal-to-noise ratio (SNR) optimization. A 9:1 beam splitter is placed after the LCM for producing a reference signal. While all subsequently captured signals are compared against this reference, any fluctuation in the characteristics of the LCM will be eliminated through a differential measurement approach. We have implemented a temperature-controlled chamber for stabilizing the temperature of the LCM to within ±0.01 °C. We found that this is very important for achieving the expected performance in the sensor system. The LCM is placed between two mirrors so that the beam gets folded a number of times and attains a larger retardation modulation depth. In the present case, the total retardation depth is 14π (i.e. going through the LCM 7 times). The significance of this technique is that we need to capture as many cycles of phase modulation as possible in every signal trace in order to increase the phase extraction accuracy as the subsequent data processing algorithm relies on comparing the phase locations of truncated sine waves. The reference beam passes through a linear polarizer at 45° off the p-polarization axis so that interference between p- and s-polarized light can provide the necessary intensity variation as we modulate the retardation using the LCM. If we take p-polarized light as the probe beam, while s-polarized light as the self-reference beam, then the system will naturally eliminate common-mode noises which are not due to the phase difference between the two polarizations (i.e. retardation). The SPR sensing head consists of two elements: a prism coupler and a

sensor chip covered by a flow-cell. The dove prism coupler is made from BK7 glass and the sensor chip is a microscope glass slide ($25 \times 25 \times 1$ (mm)) coated with a nominally 46-nm-thick gold film. Further details of the sensor chip and the flow-cell may be found from our previous paper[7]. The sensor chip is optically coupled to the back side of the dove prism through a layer of refractive index matching oil. During the experiment, sample fluid was injected into the flow-cell using a syringe. A rotational stage permits adjustment of the incident angle to around 73° so that the experiment may start at the minimum of the SPR absorption dip. At the exit of the sensor head, a photodetector collects the interference signal from the reflected beam through another polarizer positioned at 45° from the p-polarization axis. A digital storage oscilloscope (DSO, LeCroy LT264) is used for recording the signal waveforms of the probe and reference channels and the raw data will be processed with a computer. A software program has been constructed for real-time phase extraction and monitoring. The signal processing procedures include data averaging, signal trace conversion and normalization to truncated sine wave, band-pass filtering and point-wise differential phase extraction. Further details of our phase extraction algorithm may be found in our earlier paper[8]. In order to verify the performance of this system, we performed a set of refractive index measurement experiments on glycerin-water mixtures in various weight ratios.

The samples were glycerin-water mixtures with 0% to 16% of glycerin in weight percentage, which correspond to a refractive index range from 1.3330 to 1.3521[9]. Figure 2 shows the measured SPR phase versus glycerin concentration. As seen from the plot, we could only observe approximately 50% of the maximum SPR phase change across the SPR resonance dip. This is because of the fact that our experiments started at the minimum point of the SPR resonance dip, which was halfway through the phase jump across resonance. The system continuously shifted away from resonance as the refractive index of the sample increased from 1.3330 to 1.3521. In order to calculate the best resolution limit of our system, we use the first two data points from the plot, which were obtained in the most sensitive region (near the resonance dip). They correspond to two sample mixtures, i.e. pure water and 0.05% glycerin in water, with a change of refractive index equivalent to 6×10^{-5} RIU and a SPR phase change of 32.12°. Moreover, as shown in Fig. 3, the system stability was also monitored and we obtained a phase measurement fluctuation of ±0.0028° (root-mean-square) or 0.013° (peak-to-peak)

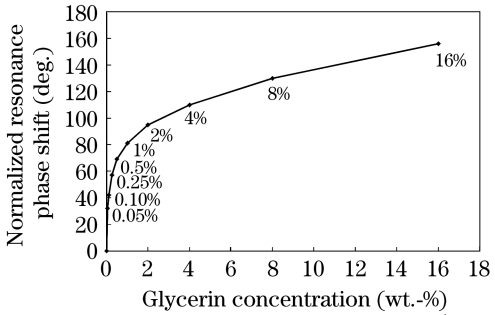

Fig. 2. Variation of relative differential phase (reference to pure water) versus glycerin concentration.

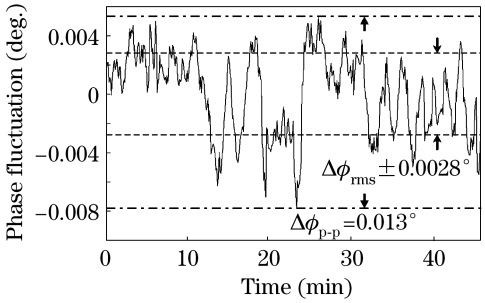

Fig. 3. System stability measurement over 45 min.

within 45 min. It should be mentioned that the stability of the present system is significantly better than that reported from a similar LCM-based setup[4], which is around 0.1° (peak-to-peak). Using ±0.0028° as the baseline of our system stability, the calculated resolution limit is $\pm 5.2 \times 10^{-9}$ RIU.

A single-beam self-referenced phase-sensitive SPR sensor based on differential measurement between the probe and reference channels has been demonstrated. In order to address the limitations of LCM as a retardation modulator, we proposed two simple techniques to improve its modulation range and stability, i.e., the use of multi-pass beam-folding device and active temperature control. Since both s- and p-polarization traverse an identical path in the system, the signal generated from direct interference between them will eliminate unwanted common-mode errors not related to the shift in SPR condition. Moreover, errors due to temperature dependence and nonlinearity of the LCM can also be removed by taking the differential change between the probe and reference signals obtained immediately before and after the sensor head. The experimental resolution limit of our system is estimated to be $\pm 5.2 \times 10^{-9}$ RIU.

The authors wish to acknowledge funding support of this project from the Research Grants Council under Competitive Earmarked Research Grant (CERG) Project No. 411906. We are also grateful to The Chinese University of Hong Kong for providing research student support to S.-Y. Wu. S.-Y. Wu's e-mail address is sywu@ee.cuhk.edu.hk.

References

1. R. L. Rich and D. G. Myszka, J. Mol. Recogn. **13,** 388 (2000).
2. S. G. Nelson, K. S. Johnston, and S. S. Yee, Sensor Actuator B **35,** 187 (1996).
3. X. Yu, L. Zhao, H. Jiang, H. Wang, C. Yin, and S. Zhu, Sensor Actuator B **76,** 199 (2001).
4. Y.-D. Su, S.-J. Chen, and T.-L. Yeh, Opt. Lett. **30,** 1488 (2005).
5. I. R. Hooper and J. R. Sambles, Appl. Phys. Lett. **85,** 3017 (2004).
6. J.-Y. Lee, H.-C. Shih, C.-T. Hong, and T. K. Chou, Opt. Commun. **278,** 283 (2007).
7. S. Y. Wu and H. P. Ho, Proc. SPIE **6099,** 60990R (2006).
8. H. P. Ho, W. C. Law, S. Y. Wu, C. Lin, and S. K. Kong, Biosens. Bioelectron. **20,** 2177 (2005).
9. R. C. Weast, (ed.) *Handbook of Chemistry and Physics* (68th edn.) (CRC Press, Boca Raton, 1987) D-232.