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An ellipsometric surface plasmon resonance sensor for the direct monitoring of small-molecular-weight analytes

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Abstract: We report on a high-sensitivity surface plasmon resonance (SPR) sensor based on phase measurements using rotating analyzer ellipsometry. In this work, the experimental setup for the SPR sensor was based on a custom-built rotating analyzer ellipsometer, which was equipped with a SPR cell and a microfluidic system. The most important feature of our system is that biomolecular detection sensitivity can be significantly enhanced by measuring the phase difference between the s- and p-polarizations. In this paper, we demonstrate how a custom-built rotating analyzer ellipsometer in the SPR condition can be used to directly detect the interaction of D-biotin and protein tyrosine phosphatase (PTP) inhibitor with immobilized streptavidin and PTP maltose binding protein (MBP)-SHP2.

Keywords: Surface Plasmon Resonance, ellipsomtry, low-molecular-weight analytes, biomolecular interactions

1. INTRODUCTION

Surface plasmon resonance (SPR) has become a leading technology in the study of biological binding events in real time [1]. Theoretically, the dispersion relation of a surface plasmon is sensitive to the refractive index (RI) of the ambient medium or to the existence of adsorbates or a dielectric layer on the surface of the metallic film [2].

Several recent studies have demonstrated improvements i n the characteristics of SPR biosensors by taking advantage of the phase-polarization properties of light that is reflected i n the context of SPR. The resultant phase shift can then be s ensitively measured in various interferometry [3-9] and pola rimetry [10-12] schemes so as to potentially provide a two o rders of magnitude gain in sensitivity.

Ellipsometry is frequently applied in biology and biochemistry, as well as to the characterization of organic layers in general [13-15]. When the SPR effect is combined with ellipsometry in a total internal reflection mode, a highly sensitive technique can be obtained [16]. Under SPR conditions, the ellipsometric phase shift significantly enhances thin film sensitivity such that more information becomes available in comparison to traditional ellipsometry or SPR techniques.

Several companies are currently manufacturing SPR instruments that are designed to study biomolecular interactions [17, 18]. Each company produces different SPR systems that are equipped with a variety of options that are applicable to specific applications. Commercial SPR biosensors are generally capable of detecting 1 pg/mm^2 of absorbed analytes. The primary problem with current commercially available SPR technology involves the existence of a physical lower detection limit (LOD) of amplitude-sensitive schemes. This limit is conditioned by the level of noise in the measurements and is normally estimated to be 10^{-6}-10^{-5} refractive index unit (RIU) [19, 20] for various sensor implementations using angular, spectral, or intensity interrogations [21].

In this paper, we present the design and application of a high-sensitivity SPR sensor that is based on the phase measurement of rotating analyzer ellipsometry. The experimental setup for this SPR sensor is based on a custom-built rotating analyzer ellipsometer that is equipped with a SPR cell and a microfluidic system. In this work, we achieved a detection limit of 0.1 pm with $1.0\text{x}10^{-7}$ RIU, which is equivalent to 0.1 pg/mm^2. The measurement capability of our setup was demonstrated by the direct monitoring of the interaction of D-biotin and PTP inhibitor with immobilized streptavidin and PTP MBP-SHP2.

2. METHODOLOGY

1. SPR ellipsometry

First, we briefly introduce the analysis of the phase of the reflected light using ellipsometry [22] under SPR conditions. In ellipsometry, two parameters, specifically ψ and Δ, are measured by applying a probe beam with a known polarization state onto a sample and then investigating the polarization state of the reflected beam. In reflection mode, the parameters ψ and Δ are given by Eq.(1),

$$\tan\psi = |r_p| / |r_s|, \quad (1)$$

and Eq.(2),

$$\Delta = \delta_p - \delta_s , \qquad (2)$$

where $r_p = \left[|r_p| \exp(i\delta_p) \right]$ and $r_s = \left[|r_s| \exp(i\delta_s) \right]$ are the complex reflection coefficients for p- and s-polarized light, respectively. The s-direction is taken to be perpendicular to the plane of incidence of the sample plane. The p-direction is parallel to the plane of incidence and normal to the s-direction.

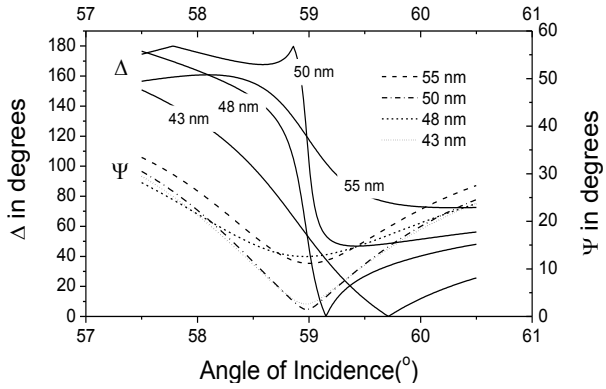

Fig. 1. Ellipsometric parameters ψ and Δ in the Kretchmann-type SPR geometry of total internal reflection.

Figure 1 depicts the effect of the gold film thickness on the resonance profile. Here, we present a particular optical configuration of an ellipsometer that consists of a fixed polarizer, sample, and rotating-analyzer. The phase shift Δ shows a very high sensitivity in the optimum condition of SPR. All calculations were performed with WVASE32 software (J. A. Woollam Company).

In our system, a gold film of about 36 to 42.4 nm in thickness near the optimum condition (39.8 nm) was used in the SPR sensor to monitor small molecule-protein interactions. The closer the thickness is to the optimal conditions, the sharper and more promising the SPR sensing characteristics. The thickness of the metal film, which not only determines the appearance of the SPR phenomenon but also directly affects the detection resolution, is a key parameter of a SPR biosensor. It is necessary to optimize the thickness of the metal film so as to obtain a high detection resolution. With an optimized metal film thickness, the precision of the SPR measurement depends on the intensity variation of the light source, the alignment of the optical system, the temperature variation of the microfluidics, and the uniformity of the metal film. Many gold films that are coated onto SF10 prisms are used to obtain the optimized thickness and refractive index of the metal film. In rotating analyzer ellipsometry, polarizer tracking, which positions the polarizer azimuth at the angle of ψ, is the best way to measure a sample with good precision. The incident light on a sample in the tracking angle of the polarizer consists of nearly p-polarized light, which results in a low reflectance signal. A stabilized laser light is focused on the sensor surface to obtain a high S/N ratio in the tracking

2. *Experimental setup*

The experimental setup for SPR ellipsometry is based on a custom-built rotating analyzer ellipsometer [23] that is equipped with an SPR cell and a microfluidic system. The experimental setup of the rotating analyzer system is illustrated in Figure 2. A stabilized laser light at 5 mW and 633 nm is used as the light source. SF10 glass prisms were coated on the bottom side with 40 nm of gold (SPR layer) via vapor deposition. Outside of the metal layer, the liquid cell was designed for SPR measurements under flow injection conditions. The microfluidic system consists of two channels and valves.

The measurement capability of our setup was demonstrated by the direct monitoring of the binding kinetics of analytes (D-biotin, PTP inhibitor) with immobilized ligand (streptavidin, PTP MBP-SHP2). As a transparent ultrathin dielectric layer, 11-mercaptoundecanoic acid maleimide (MUAM) and 11-mecaptoundecanoic acid (MUA) were deposited onto the surface of the gold thin film. The sensor was calibrated with an ethanol/water solution (1.5%), and then 12 μg/ml streptavidin and 0.15 mg/ml PTP MBP-SHP2 in PBS were flowed over the sensor surface at 20 μl/min. The surface was then washed with PBS until it stabilized. Finally, D-biotin (Sigma–Aldrich) and PTP inhibitor V, PHPS1 ($C_{21}H_{15}N_5O_6S$, 465.4 Da, Santa cruze) in PBS were flowed over the sensor surface at 100 μl/min, and the subsequent binding events were measured.

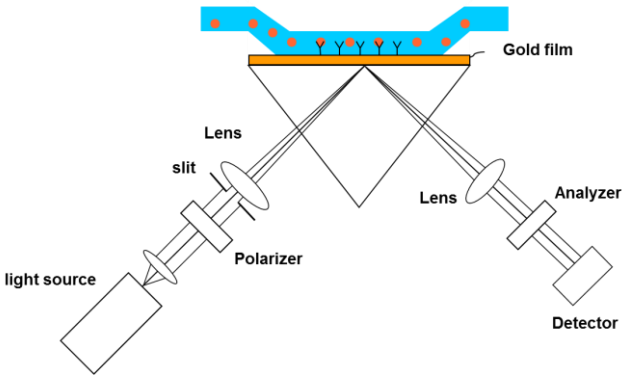

Fig. 2. Schematics of the SPR ellipsometry instrument that was designed for the characterization of small-molecule interactions

3. RESULTS AND DISCUSSION

We investigated the thickness and refractive index of the gold film that was used in the SPR sensor. The plots in Figure 3 depicts the ellipsometric parameters that were experimentally obtained by the custom-built rotating analyzer ellipsometer. The sharp reflectivity minimum is due to SPR and is very sensitive to the refractive index of the surrounding medium or the existence of adsorbates or the dielectric layer at the surface of the gold film. The symbols indicate the experimental data for the gold thin film,

whereas the solid lines are the best fit to the optical modeling. The model consists of three media: SF10 glass as an ambient, a thin gold layer, and a water substrate. Optical data for the involved materials were taken from the literature: SF10 (from SCHOTT) and water (from the SOPRA data bank). The sharp reflectivity minimum was very sensitive to the thickness of the gold thin film. We obtained the thicknesses and complex refractive indices of thin gold films from the best fit results. The fitting parameters are tabulated in Table 1. The solid lines are the calculated curves using the parameters in Table 1, which were obtained from the ellipsometric model. Table 1 demonstrates that the absorption coefficient values of gold films that were obtained from experimental data were much higher extinction coefficient than those of the gold data (0.19683+3.0905i) in Palik's handbook [24].

The sensor was calibrated with a reference solution of ethanol/DDW (double-distilled water). Fig. 4 depicts the ellipsometric phase difference of the SPR sensors with ethanol (1.5%)/DDW. The results of calibration, which are shown in Figure 5, indicate that the phase change, $\delta\Delta$, had an almost linear response to the concentration of ethanol in the double-distilled water solutions.

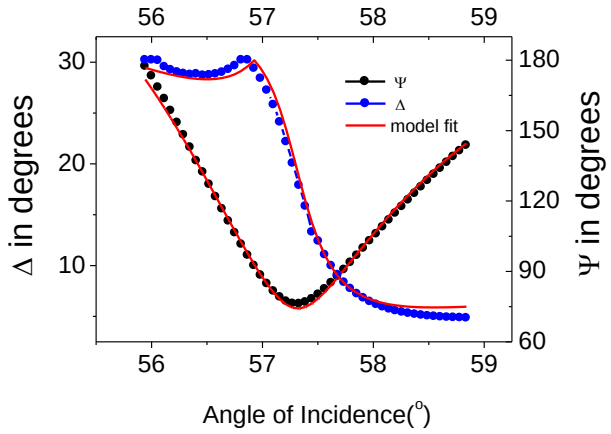

Fig. 3. The profiles of the ellipsometric parameters, ψ and Δ. The symbols indicate the experimental data, whereas the solid lines are the best fits to the theoretical model. The fitting parameters are indicated in Table 1.

Table 1. The refractive indexes and thicknesses of each layer(λ =632.8 nm)

Material	Refractive index		Thickness (nm)
	n	K	
SF10	1.7231	0	-
gold film	0.20-0.35	3.42-3.44	42-50
water	1.333	0	-

We obtained a very small standard deviation of 0.001° in the ellipsometric phase difference, which is equivalent to 0.1 pg/mm^2. In the phase measurement based on the polarimetry method, instrument resolutions of 0.02° and 0.007° have been reported by R. Naraoka et al. [10] and S. Patskovsky et al. [12], respectively. In the phase measurement based on the heterodyne method, instrument resolutions of 0.025° and 0.01° have been reported by Nelson et al. [3] and Xinglong et al. [5].

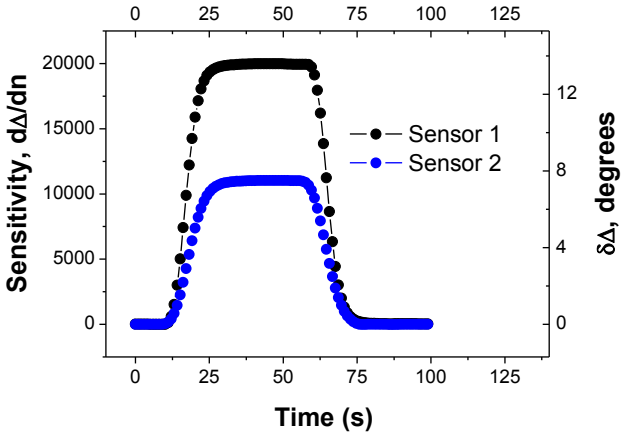

Fig. 4. Sensitivity and response curves. Ellipsometric phase differences with the injection of ethanol/double-distilled water solutions (1.5%) for SPR sensors with two gold film thicknesses

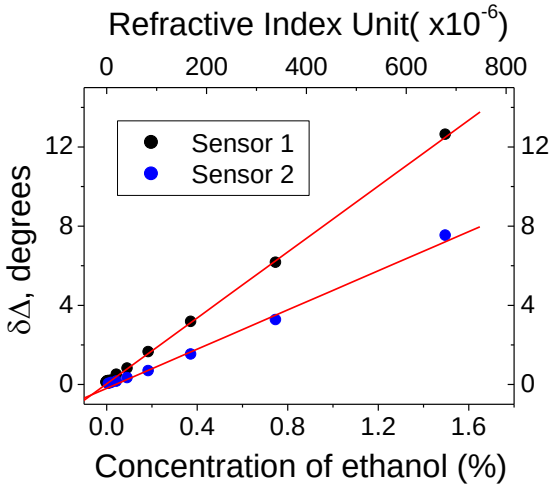

Fig. 5. Calibration curve for the response of the phase change, $\delta\Delta$, as a function of the concentration of ethanol in double-distilled water solutions.

Figure 6 depicts the process flow diagram from the sensor surface to the binding of D-biotin. The 12-µg/ml streptavidin (Sigma-Aldrich) in PBS on the biotinylated sensor surface was flowed over the sensor surface at a rate of 20 µl/min. Figure 7 depicts the binding kinetics of D-biotin to immobilized streptavidin. The immobilization procedure yielded approximately 3700x10^{-6} RIU of streptavidin. D-biotin was injected for 2 min at concentrations of 15 nM, 122 nM, and 250 nM in the same channel of the immobilized sensor chip. During the PBS

wash phase, a little loss of material was observed, as shown in Fig. 7(b).

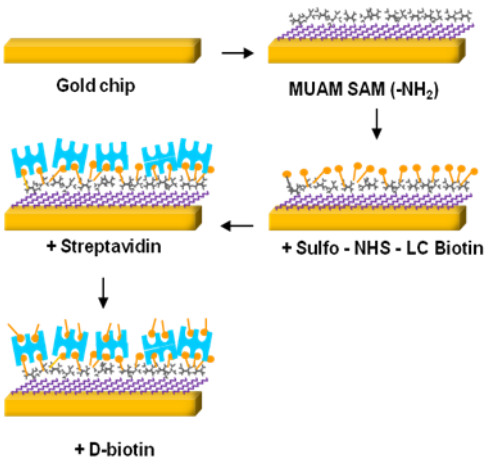

Fig. 6. The process flow diagram (MUAM SAM, biotinylated surface, the immobilization of streptavidin, and the injection of D-biotin).

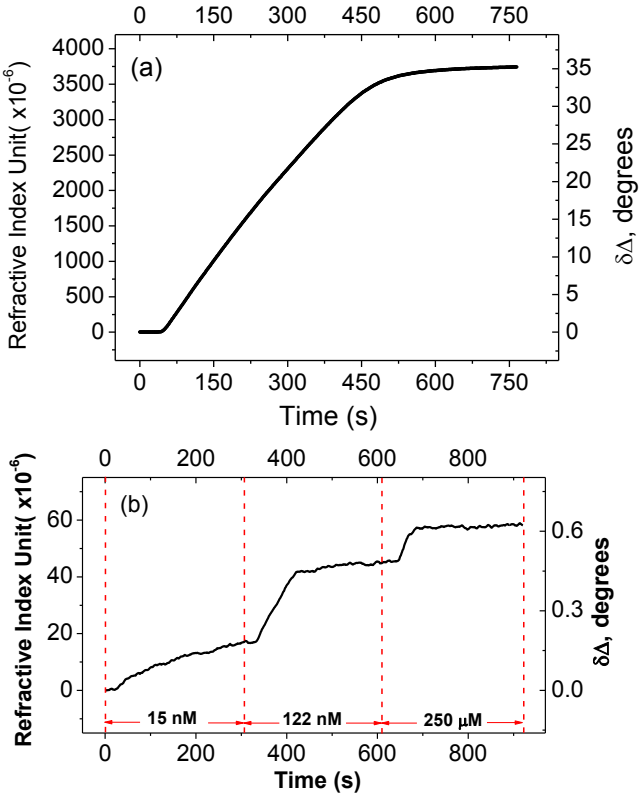

Fig. 7. Ellipsometric phase difference and refractive index variation for the measurement of biomolecular interactions: (a) the immobilization of streptavidin on a biotinylated surface and (b) the injection of D-biotin to immobilized streptavidin.

Figure 8 depicts ellipsometric phase differences and refractive index variation with the injection of ethanol/double-distilled water solutions (1.5%) after the interaction of D-biotin with immobilized streptavidin.

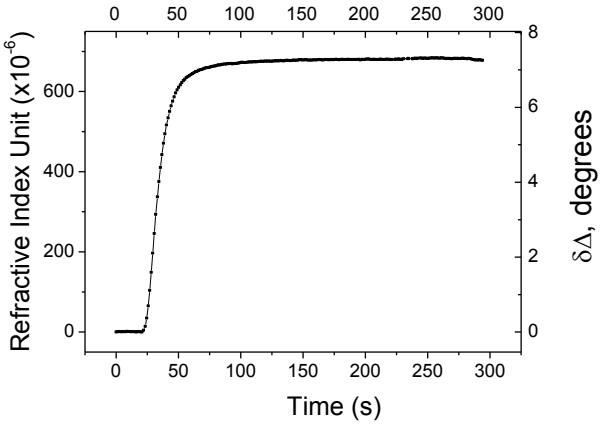

Fig. 8. Ellipsometric phase differences and refractive index variation with the injection of ethanol/double-distilled water solutions (1.5%) after the interaction of D-biotin with immobilized streptavidin.

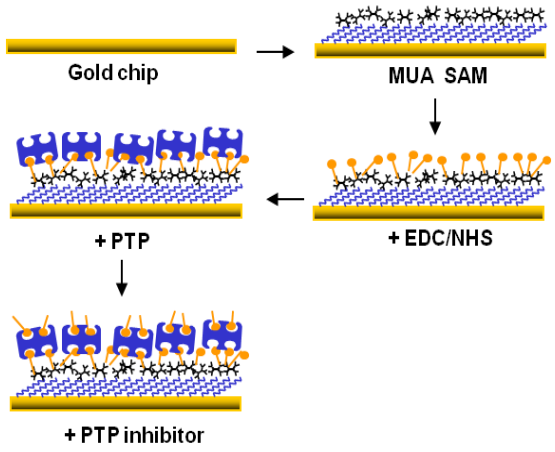

Fig. 9. The process flow diagram (MUA SAM, the immobilization of PTP MBP-SHP2, and the injection of PTP inhibitor).

Figure 9 depicts the process flow diagram from the sensor surface to the binding of PTP inhibitor. The 0.15 mg/ml PTP MBP-SHP2 in PBS on the activated sensor surface was flowed over the sensor surface at a rate of 20 µl/min. Figure 10 depicts the binding kinetics of PTP inhibitor V, PHPS1($C_{21}H_{15}N_5O_6S$, 465.4 Da, Santa cruze) to immobilized PTP MBP-SHP2. The immobilization procedure yielded approximately $3000x10^{-6}$ RIU of PTP MBP-SHP2. PTP inhibitor was injected for 1 min at concentrations of 400 nM and 600 nM in the same channel of the immobilized sensor chip. During the PBS wash phase, a steady loss of material was observed, as shown in Fig. 10(b).

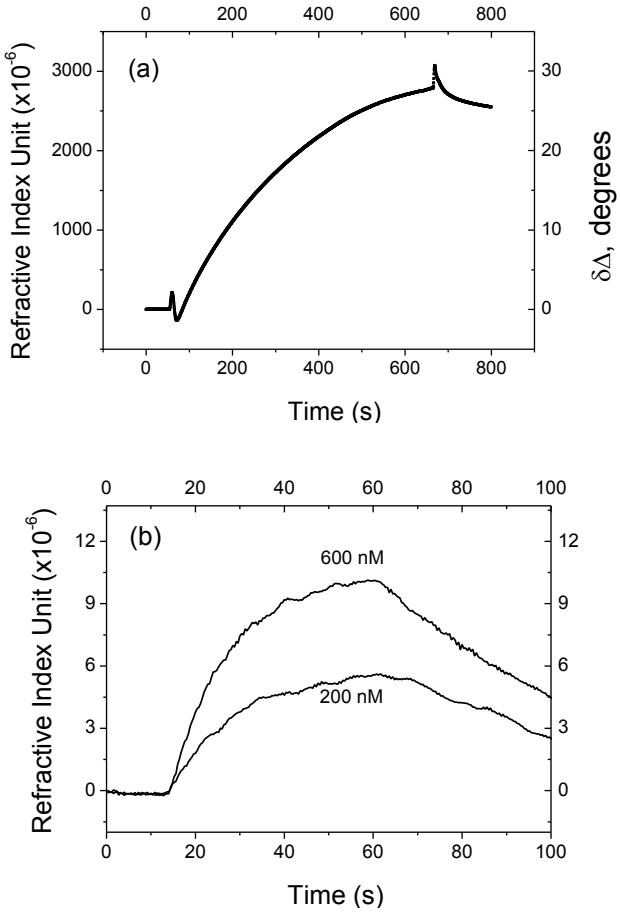

Fig. 10. Ellipsometric phase difference and refractive index variation for the measurement of biomolecular interactions: (a) the immobilization of PTP and (b) the injection of PTP inhibitor.

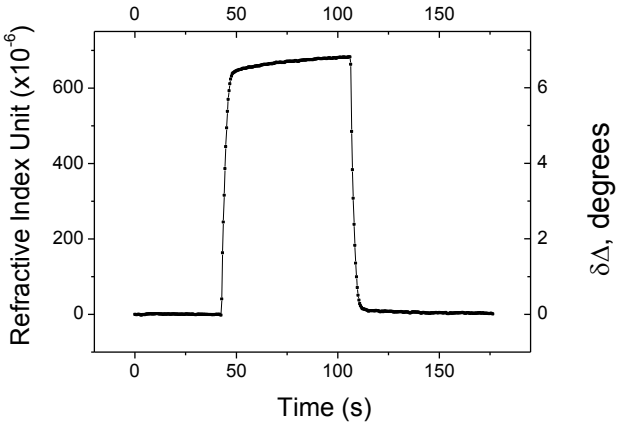

Fig. 11. Ellipsometric phase differences and refractive index variation with the injection of ethanol/double-distilled water solutions (1.5%) after the interaction of PTP inhibitor with immobilized PTP maltose binding protein (MBP)-SHP2.

Figure 11 depicts ellipsometric phase differences and refractive index variation with the injection of ethanol/double-distilled water solutions (1.5%) after the interaction of PTP inhibitor with immobilized PTP maltose binding protein (MBP)-SHP2.

4. CONCLUSIONS

We achieved a detection limit of 0.1 pm with 1.0 x 10^{-7} RIU, which is equivalent to 0.1 pg/mm^2. In this paper, we demonstrate how a custom-built rotating analyzer ellipsometer in the SPR condition can be used to directly detect the interaction of low-molecular-weight analytes (D-biotin, 244 Da and PTP inhibitor, 465.4 Da) with immobilized streptavidin and PTP MBP-SHP2.

In the drug discovery process, low-molecular-weight (200-600 Da) compounds are routinely tested for binding to a wide variety of receptors and early lead candidates. In particular, to monitor the interaction of small molecule-protein, it is necessary to design a high-sensitivity SPR sensor with a sensitivity of greater than 1 pg/mm^2. Our sensor demonstrates a much better sensitivity in comparison to other SPR sensors based on reflectometry or phase measurements.

5. REFERENCES

[1] B. Liedberg, C. Nylander, and I. Lundstrom, "Biosensing with surface plasmon resonance - how it all started, " Biosens. Bioelectron. **10**, 1-9 (1995).

[2] J. Homola, "Present and future of surface plasmon resonance biosensors," Anal. Bioanal. Chem. **377,** 528-539 (2003).

[3] S.G. Nelson, K.S. Johnston and S.S. Yee, "High sensitivity surface plasmon resonance sensor based on phase detection," Sens. Actuators B **35/36**, 187-191 (1996).

[4] H. P. Ho, W. W. Lam, and S. Y. Wu, "Surface plasmon resonance sensor based on the measurement of differential phase," Rev. Sci. Instrum. **73**, 3534–3539 (2002).

[5] Y. Xinglong, W. Dingxin, and Y. Zibo, "Simulation and analysis of surface plasmon resonance biosensor based on phase detection," Sens. Actuators B **91**, 285–290 (2003).

[6] Y.-D. Su, S.-J. Chen, and T.-L. Yeh, "Common-path phase-shift interferometry surface plasmon resonance imaging system," Opt. Lett. **30**, 1488–1490 (2005).

[7] W. C. Kuo, C. Chou, and H. T. Wu, "Optical heterodyne surface-plasmon resonance biosensor," Opt. Lett. **28**, 1329–1331 (2003).

[9] Y.-C. Li, Y.-F. Chang, L.-C. Su, and C. Chou, "Differential-phase surface plasmon resonance biosensor," Anal. Chem. **80**, 5590–5595 (2008).

[10] R. Naraoka, and K. Kajikawa, "Phase detection of surface plasmon resonance using rotating analyzer method," Sens. Actuators B **107**, 952–956 (2005).

[11] P. P. Markowicz, W. C. Law, A. Baev, P. Prasad, S. Patskovsky, and A. V. Kabashin, "Phase-sensitive time modulated SPR polarimetry for wide dynamic range biosensing," Opt. Express **15**, 1745-1754 (2007).

[12] S. Patskovsky, M. Maisonneuve, M. Meunier, and A. V. Kabashin, "Mechanical modulation method for ultrasensitive phase measurements in photonics biosensing," Opt. Express **16**, 21305–21314 (2008).

[13] H. Elwing, "Protein absorption and ellipsometry in biomaterial research," Biomaterials **19,** 397-406 (1998).

[14] P. Tengvall, I. Lundström and B. Liedberg, "Protein adsorption studies on model organic surfaces: an ellipsometric and infrared spectroscopic approach," Biomaterials **19,** 407-422 (1998).
[15] H. Arwin, "Spectroscopic ellipsometry and biology: recent developments and challenges," Thin Solid Films **313**/**314**, 764-774 (1998).
[16] H. Arwin, M. Poksinski, and K. Johansen, "Total Internal Reflection Ellipsometry Principles and Applications," Appl. Opt. **43**, 3028-3036 (2004).
[17] E. Zubritsky, "New choices for SPR-emerging products are filling niches in the SPR market," Anal. Chem. **72**, 289A–292A (2000).
[18] C.L. Baird, D.G. Myszka, "Current and emerging commercial optical biosensors," J. Mol. Recog. **14**, 261–268 (2001).
[19] X.D. Hoaa, A.G. Kirk b, M. Tabrizian, "Towards integrated and sensitive surface plasmon resonance biosensors: A review of recent progress," Biosens. Bioelectron. **23**, 151–160 (2007).
[20] Andrei V. Kabashin, Sergiy Patskovsky, Alexander N. Grigorenko, "Phase and amplitude sensitivities in surface plasmon resonance bio and chemical sensing," Opt. Express **17**, 21191-21203 (2009).
[21] R. B. M. Schasfoort, and A. J. Tudos, eds., *Handbook of Surface Plasmon Resonance* (RSC, Cambridge, UK, 2008).
[22] R.M.A. Azzam and N.M. Bashara, *Ellipsometry and Polarized Light* (North-Holland, Amsterdam, 1977).
[23] H. M. Cho, W. Chegal, Y. J. Cho, J. M. Won, H. M. Lee, and Jae Heung Job "Kinetic analysis of biomolecular interactions by surface plasmon enhanced ellipsometry", Proc. of SPIE Vol. 8099 80990U-1(2011).
[24] David W. Lynch and W. R. Hunter, "Gold (Au)," in *Handbook of Optical Constants of Solids*, E. D. Palik, ed. (Academic, Orlando, Fla., 1985).