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Detection of Prostate-Specific Antigen with a Paired Surface Plasma Wave Biosensor

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In this study, we demonstrated that an amplitude-sensitive paired surface plasma wave biosensor (PSPWB) is capable of real-time detection of prostate-specific antigen (PSA) in diluted human serum without labeling. Experimentally, the detection limit of PSPWB was 8.4×10^{-9} refractive index unit (RIU) and the PSPWB could measure PSA in a phosphate buffered saline solution from 10 fg/mL (~300 aM) to 100 pg/mL (~3 pM) successfully, with demonstration of a linear relationship between PSA concentrations and surface plasmon resonance (SPR) signals. Therefore, results were obtained over a wide dynamic range 5 orders of magnitude for analyte concentration. In addition, the PSPWB successfully detected PSA in diluted human serum as well. These experimental results indicate that the PSPWB is capable of detection with high sensitivity over a wide range by using SPR-based biosensors and has a capability of detecting biological analytes in clinical sample without complicated operating procedures.

Surface plasmons (SPs) have been investigated since the 1950s.[1,2] SPs can be described as the collective oscillation of free electrons on the surface of a material, such as gold or silver, at the interface with a dielectric medium. The electromagnetic fields at the surface penetrate into the dielectric medium only to a depth of approximately 200 nm.[3−6] The most common technique to excite SPs is the use of evanescent waves generated by an incident laser beam undergoing attenuated total reflection (ATR).[3,7,8] Because the evanescent waves satisfy the phase-matching condition with surface plasmon waves (SPWs) excited on the metal/dielectric interface, the maximum coupling between these two waves takes place while SPWs are in resonance.[3,8] Such a surface phenomenon is highly sensitive to changes in the effective refractive index of the test medium in the vicinity of the metal surface. Consequently, surface plasmon resonance (SPR) is a powerful optical technology for the detection of biological analytes and the monitoring of biomolecular interactions in real time without chemical labeling.[2,9−14] However, it is a challenge for conventional SPR biosensors to detect an analyte at low concentrations or with low molecular weight because of sensitivity limitations.[9] The detection limit is about 1−10 nM for molecules with low molecular weight, and it is even higher for smaller molecules.[9,15,16] One way to improve the sensitivity of SPR biosensors, therefore, is to increase the mass concentration in the interaction region of SPW, resulting in the enhancement of SPR signals.[9] For example, the immunoassay based on the conjunction of gold nanoparticles and antibodies was proposed to amplify SPR signals.[17−19] An alternative method that can improve the sensitivity of a SPR system is using fluorescence labeling, which is referred to as surface plasmon field-enhanced fluorescence spectroscopy (SPFS).[20−22] However, this method is not label-free. Recently,

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(1) Ritchie, R. H. *Phys. Rev.* **1957**, *106*, 874–881.
(2) Chiang, Y. L.; Lin, C. H.; Yen, M. Y.; Su, Y. D.; Chen, S. J.; Chen, H. F. *Biosens. Bioelectron.* **2009**, *24*, 1905–1910.
(3) Stark, P. R. H.; Halleck, A. E.; Larson, D. N. *Methods* **2005**, *37*, 37–47.
(4) Law, W. C.; Markowicz, P.; Yong, K. T.; Roy, I.; Baev, A.; Patskovsky, S.; Kabashin, A. V.; Ho, H. P.; Prasad, P. N. *Biosens. Bioelectron.* **2007**, *23*, 627–632.
(5) Shan, X.; Huang, X.; Foley, K. J.; Zhang, P.; Chen, K.; Wang, S.; Tao, N. *Anal. Chem.* **2010**, *82*, 234–240.
(6) Bozhevolnyi, S. I.; Erland, J.; Leosson, K.; Skovgaard, P. M. W.; Hvam, J. M. *Phys. Rev. Lett.* **2001**, *86*, 3008–3011.
(7) Seidel, J.; Grafstrom, S.; Eng, L. *Phys. Rev. Lett.* **2005**, *94*, 177401.
(8) Sharma, A. K.; Jha, R.; Gupta, B. D. *IEEE Sens. J.* **2007**, *7*, 1118–1129.
(9) Cao, C.; Kim, J. P.; Kim, B. W.; Chae, H.; Yoon, H. C.; Yang, S. S.; Sim, S. J. *Biosens. Bioelectron.* **2006**, *21*, 2106–2113.
(10) Wang, S.; Forzani, E. S.; Tao, N.; et al. *Anal. Chem.* **2007**, *79*, 4427–4432.
(11) VanWiggeren, G. D.; Bynum, M. A.; Ertel, J. P.; Jefferson, S.; Robotti, K. M.; Thrush, E. P.; Baney, D. M.; Killeen, K. P. *Sens. Actuators, B: Chem.* **2007**, *127*, 341–349.
(12) Hoa, X. D.; Kirk, A. G.; Tabrizian, M. *Biosens. Bioelectron.* **2007**, *23*, 151–160.
(13) Boozer, C.; Kim, G.; Cong, S.; et al. *Curr. Opin. Biotechnol.* **2006**, *17*, 400–405.
(14) Yuan, W.; Ho, H.-P.; Wu, S. Y.; Suen, Y. K.; Kong, S. K. *Sens. Actuators, A: Phys.* **2009**, *151*, 23–28.
(15) Petz, M. *Monatsh. Chem.* **2009**, *140*, 953–964.
(16) Gomes, P.; Andreu, D. *J. Immunol. Methods* **2002**, *259*, 217–230.
(17) Lyon, L. A.; Musick, M. D.; Natan, M. J. *Anal. Chem.* **1998**, *70*, 5177–5183.
(18) Lee, W.; Oh, B.-K.; Kim, Y.-W.; Choi, J.-W. *J. Nanosci. Nanotechnol.* **2006**, *6*, 3521–3525.
(19) Ko, S.; Park, T. J.; Kim, H.-S.; Kim, J.-H.; Cho, Y.-J. *Biosens. Bioelectron.* **2009**, *24*, 2592–2597.
(20) Liebermann, T.; Knoll, W. *Colloid Surf., A: Physicochem. Eng. Asp.* **2000**, *171*, 115–130.

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another category of SPR biosensors, used in combination with an interferometer technique that includes both the spatial and temporal domains,[23−30] has been shown to improve detection limits of SPR systems. In a previous study, we presented an amplitude-sensitive, paired surface plasma wave biosensor (PSPWB) based on SPR in conjunction with an optical heterodyne technique.[25,27] The feature of this biosensor is the existence of two highly correlated SPWs with different temporal frequencies on the metal/dielectric interface. Experimentally, we verified that the PSPWB not only exhibits higher sensitivity but also provides a wider dynamic range for measuring effective refractive index variation.

The present study reports on using the PSPWB to further improve the sensitivity of real-time prostate-specific antigen (PSA) detection as compared to detection using conventional methods. According to the World Health Organization (WHO), prostate cancer (PCa) is a common malignancy and a top 10 leading cause of cancer deaths in the male population. PSA has been recognized as the premier tumor marker for the detection of early stage PCa and for monitoring the recurrence of the disease after treatment.[31] The use of PSA testing can help detect prostate tumors while still small in size, low-grade, and localized.[32] PSA is a monomeric protein of 237 amino acid residues, including 4 glycosylation sites.[33] It has two predominant forms in human serum: either free PSA (f-PSA) or PSA complexed to α-1-antichymotrypsin (PSA-ACT). Total PSA (t-PSA) refers to the sum of f-PSA and PSA-ACT.[34] The PSA concentration in healthy males is usually less than 4 ng/mL,[34] while PSA levels above 100 ng/mL in men's blood is a highly probable indication of PCa.[31] In previous research, PSA assays were usually based on immunoassays.[33,35–42] Normally, such studies propose that the sensitivity of PSA detection in buffer is about several picograms per milliliter. On the other hand, SPR-based immunosensors have shown that integration with gold nanoparticles successfully increased the sensitivity of PSA detection to 10 fM (∼330 fg/mL) in buffer,[41,43] and the detection limit of PSA concentrations with the SPFS-oriented method was estimated to be ∼80 fM (∼2.64 pg/mL).[44] In addition, localized SPR-based detection utilizes a nanoplasmonic sensing platform to detect PSA concentration down to 250 fM (∼8.1 pg/mL).[45]

In our study, an optical heterodyne technique integrated with a SPR biosensor, which senses both the amplitude and phase degree of the heterodyne signal in the temporal domain, has demonstrated a capability of detecting PSA concentration levels at 61 pg/mL (∼2 pM) in diluted human serum and 10 fg/mL (∼300 aM) in a phosphate buffered saline solution (PBS) in real time without labeling. The PSPWB has the advantage of synchronized detection and higher sensitivity. While making measurements, two lock-in amplifiers were used to synchronously detect the optical heterodyne signals and, in addition, the use of the amplitude ratio algorithm was adopted to significantly improve the sensitivity of the detected signal experimentally.

EXPERIMENTAL SECTION

Materials. SPRgold substrate (G chip) was purchased from Gentel Biosciences, Inc. (Madison, WI). The sensor chip CM5 (CM5 chip), immobilization buffer (10 mM sodium acetate, pH 5.0), amine coupling kit, and glycine 2.5 were purchased from Biacore, Inc. (Uppsala, Sweden). The amine coupling kit contained 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), and 1.0 M ethanolamine-HCl, pH 8.5 (ETH). Sucrose and PBS were purchased from Sigma (Saint Louis, MO). Monoclonal capture antibody (anti-t-PSA) and target antigen (t-PSA) were purchased from Meridian Life Science, Inc. (Saco, ME). Human serums were obtained from the Division of Serology & Immunology, Taipei City Hospital (Taipei, Taiwan). All chemicals were used without further purification.

Effective Refractive Index Variation (Δn_{eff}) Measurement. The G chip with a reaction area of 80 mm^2 and a thickness of 47.5 nm was utilized for measuring the change in the effective refractive index of the sucrose−water solution. The thickness was not a key factor in the experiment. Different wt % concentrations of sucrose−water solutions, i.e., 0 wt %, 0.000 01 wt %, 0.000 03 wt %, and 0.000 05 wt % in 1 mL volume each, were prepared separately. The wt % concentration is defined by the weight of the solute divided by the weight of the solution multiplied by 100%. Each sucrose−water solution was injected into the reaction chamber to pass over the G chip surface for several minutes.

Immobilization of the Capture Antibody. The capture antibody was covalently immobilized to the dextran matrix on a CM5 chip according to the amine-coupling protocol provided by Biacore, Inc. The protocol for this method is as follows: (1) The CM5 chip was activated by immersing it in the amine coupling

(21) Yu, F.; Yao, D.; Knoll, W. *Anal. Chem.* **2003**, *75*, 2610–2617.
(22) Ekgasit, S.; Thammacharoen, C.; Yu, F.; Knoll, W. *Anal. Chem.* **2004**, *76*, 2210–2219.
(23) Yuan, W.; Ho, H.-P.; Wong, C. L.; Kong, S. K.; Lin, C. *IEEE Sens. J.* **2007**, *7*, 70–73.
(24) Brandenburg, A.; Krauter, R.; Künzel, C.; Stefan, M.; Schulte, H. *Appl. Opt.* **2000**, *39*, 6396–6405.
(25) Kuo, W.-C.; Chou, C.; Wu, H.-T. *Opt. Lett.* **2003**, *28*, 1329–1331.
(26) Wu, S.-Y.; Ho, H.-P.; Law, W.-C.; Lin, C.; Kong, S. K. *Opt. Lett.* **2004**, *29*, 2378–2380.
(27) Chou, C.; Wu, H.-T.; Huang, Y.-C.; Kuo, W.-C.; Chen, Y.-L. *Opt. Express* **2006**, *14*, 4307–4315.
(28) Li, Y.-C.; Chang, Y.-F.; Su, L.-C.; Chou, C. *Anal. Chem.* **2008**, *80*, 5590–5595.
(29) Yuan, W.; Ho, H. P.; Suen, Y. K.; Kong, S. K.; Lin, C. *Appl. Opt.* **2007**, *46*, 8068–8073.
(30) Ho, H. P.; Yuan, W.; Wong, C. L.; Wu, S. Y.; Suen, Y. K.; Kong, S. K.; Lin, C. *Opt. Commun.* **2007**, *275*, 491–496.
(31) Kingsmore, S. F. *Nat. Rev. Drug Discovery* **2006**, *5*, 310–321.
(32) Lilja, H.; Ulmert, D.; Vickers, A. J. *Nat. Rev. Cancer* **2008**, *8*, 268–278.
(33) Wang, J.; Liu, G.; Wu, H.; Lin, Y. *Small* **2008**, *4*, 82–86.
(34) Panini, N. V.; Messina, G. A.; Salinas, E.; Fernández, H.; Raba, J. *Biosens. Bioelectron.* **2008**, *23*, 1145–1151.
(35) Acevedo, B.; Perera, Y.; Ruiz, M.; Rojas, G.; Benítez, J.; Ayala, M.; Gavilondo, J. *Clin. Chim. Acta* **2002**, *317*, 55–63.
(36) Chan, D. W.; Bruzek, D. J.; Oesterling, J. E.; Rock, R. C.; Walsh, P. C. *Clin. Chem.* **1987**, *33*, 1916–1920.
(37) Lin, J.; Ju, H. *Biosens. Bioelectron.* **2005**, *20*, 1461–1470.
(38) Okuno, J.; Maehashi, K.; Kerman, K.; Takamura, Y.; Matsumoto, K.; Tamiya, E. *Biosens. Bioelectron.* **2007**, *22*, 2377–2381.
(39) Briman, M.; Artukovic, E.; Zhang, L.; Chia, D.; Goodglick, L.; Gruner, G. *Small* **2007**, *3*, 758–762.
(40) Lee, J. H.; Hwang, K. S.; Park, J.; Yoon, K. H.; Yoon, D. S.; Kim, T. S. *Biosens. Bioelectron.* **2005**, *20*, 2157–2162.
(41) Cao, C.; Li, X.; Lee, J.; Sim, S. J. *Biosens. Bioelectron.* **2009**, *24*, 1292–1297.
(42) Grubisha, D. S.; Lipert, R. J.; Park, H. Y.; Driskell, J.; Porter, M. D. *Anal. Chem.* **2003**, *75*, 5936–5943.
(43) Choi, J. W.; Kanga, D.-Y.; Jang, Y. H.; Kim, H.-H.; Min, J.; Oh, B.-K. *Colloid Surf., A: Physicochem. Eng. Asp.* **2008**, *313−314*, 655–659.
(44) Yu, F.; Persson, B.; Lofas, S.; Knoll, W. *Anal. Chem.* **2004**, *76*, 6765–6770.
(45) Chen, S.; Svedendahl, M.; Kall, M.; Gunnarsson, L.; Dmitriev, A. *Nanotechnology* **2009**, *20*, 434015.

kit solutions, which included 0.2 M EDC and 0.05 M NHS, for about 10 min. (2) The capture antibody, at a concentration of 39 μg/mL prepared in immobilization buffer, was immobilized via reaction of its nucleophilic groups and then incubated with the sensor chip surface. This process was carried out for about 25 min. (3) The excess esters were deactivated by use of ETH, which also obviated the loosely bound protein. This process was carried out for 8 min.

Target Antigen Measurement. The CM5 chip with a reaction area of 7×7 mm^2 was prepared using the method described previously. Different concentrations of the t-PSA, i.e., 0 fg/mL, 10 fg/mL, 100 fg/mL, 1 pg/mL, and 10 pg/mL in 1 mL volume each, were prepared in PBS solution separately and then were injected into the reaction chamber to interact with the immobilized anti-t-PSA to form the ⟨anti-t-PSA/t-PSA⟩ complex on the CM5 chip surface. In addition, we utilized a 200-fold diluted human serum in 1 mL volume instead of target antigen solutions. The experimental procedure as described above was followed.

Measurements Using the PSPWB. In the PSPWB system, a polarized common-path interferometer was used.[27] A laser beam was formed with orthogonal polarization waves, which were composed of a pair of P polarized waves (TM wave, P_1 and P_2) and a pair of S polarized waves (TE wave, S_1 and S_2). Both P polarized and S polarized wave pairs had the same beat frequency ($\Delta\omega = \omega_1 - \omega_2$) for the heterodyne signals. The laser beam was then incident onto a SPR device, and a pair of SPWs was excited simultaneously by the P polarized waves at the gold/dielectric interface via ATR. Meanwhile, the paired S polarized waves propagating along a common path with the paired P polarized waves were totally reflected by the SPR device. A polarized beam splitter (PBS) was used to separate the reflected P polarized and S polarized waves. Subsequently, two temporally correlated attenuated P polarized waves (P_1' and P_2') were detected by a photodetector as signals. Similarly, two temporally correlated S polarized waves (S_1' and S_2') were detected by another photodetector as reference. Two lock-in amplifiers (Stanford Research Systems, Inc., Sunnyvale, CA) were used for simultaneous measurements

RESULTS AND DISCUSSION

System Stability of PSPWB. In order to verify that the amplitude instability caused by the excess noise of the laser beam can be reduced significantly by the amplitude normalization using the reference beam (S polarized waves), the stability of the PSPWB was recorded as the SPR signal by testing tridistilled water when the incident angle of the laser beam was near the SPR angle. The SPR signal was calculated by subtracting the mean value of the detected SPR reflectivity from each SPR reflectivity. The SPR reflectivity was obtained by measuring the optical heterodyne signal of the reflected P polarized waves (P_1' and P_2') normalized by measuring the optical heterodyne signal of the S polarized waves (S_1' and S_2'). Therefore, the unit of the detected SPR reflectivity and SPR signal is defined as an arbitrary unit (a.u.). Figure 1 shows the SPR signal stability of PSPWB spanning 10 min, and the fluctuation was around ±0.0006 au in this experiment.

Effective Refractive Index Variation (Δn_{eff}) Measurement. Different wt % concentrations of sucrose−water solution, i.e., 0 wt %, 0.000 01 wt %, 0.000 03 wt %, and 0.000 05 wt %, were injected into the reaction chamber to pass over the G chip surface for several minutes, and then an equilibrium state was reached. Figure 2 shows that a linear relation between the detected SPR signals and sucrose concentrations over the range 0−0.000 05 wt % was clearly demonstrated. The correlation coefficient (R^2) was 0.9831, and the error bar was defined as 1 standard deviation for each measurement. The SPR signal was calculated by subtracting the background level from the equilibrium level of each SPR reflectivity. The tridistilled water was treated as zero concentration in solution (0 wt % of sucrose−water solution) to define the background level in this experiment. The signal-to-noise ratio (SNR ≥ 5) was defined at different wt % concentrations of a sucrose−water solution in this experiment, and it was calculated as the detected SPR signal divided by the standard deviation of the measurement.

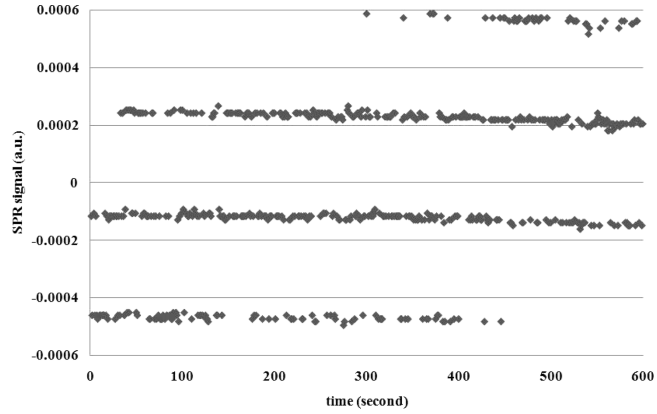

Figure 1. SPR signal stability by testing tridistilled water spanning 10 min.

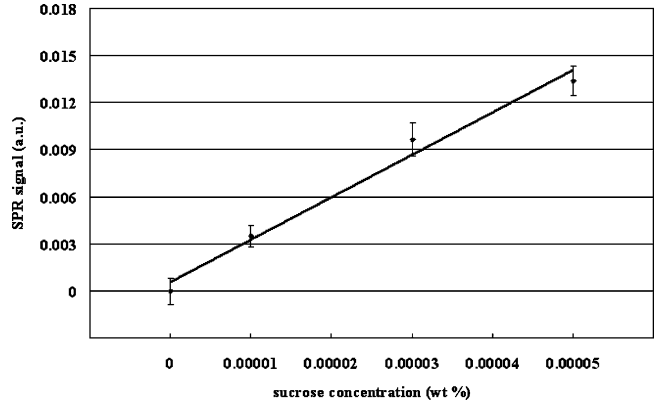

Figure 2. The linear relationship ($R^2 = 0.9831$) of sucrose−water solution concentrations versus SPR signals. The error bar is equivalent to 1 standard derivation in this experiment.

According to the table of refractive index related to wt% concentrations of the sucrose−water solution, the concentration at 0.000 01 wt % corresponds to $\Delta n_{eff} = 1.4 \times 10^{-8}$ refractive index unit (RIU) relative to pure water.[28] SNR is greater or equal to 5 at the 0.000 01 wt % sucrose−water solution, shown in Figure 2, which indicates that the detection limit of the PSPWB is $\Delta n_{eff} = 8.4 \times 10^{-9}$ RIU (SNR = 3). In contrast, the detection limits for effective refractive index variation were reported as 1×10^{-5}−1×10^{-6},[12] 4×10^{-6},[10] 2×10^{-7},[23] and 5×10^{-8} RIU[26] using different methods. Only the differential-phase SPR biosensor, which performed at 2.8×10^{-9} RIU,[28] is better

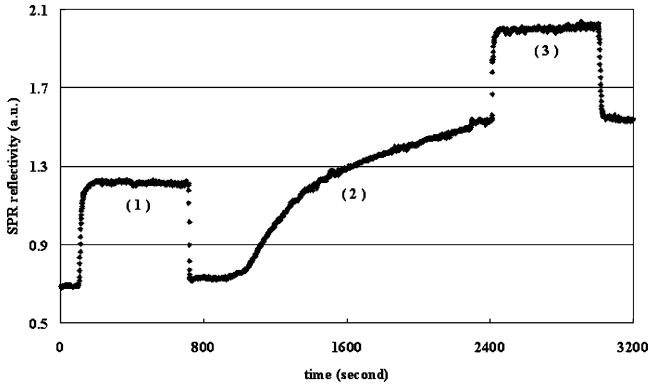

Figure 3. Sensorgram of anti-t-PSA (capture antibody) immobilized on the CM5 chip. (1), (2), and (3) represent the NHS/EDC activation, the anti-t-PSA (39 μg/mL) immobilization, and the deactivation by ETH, respectively.

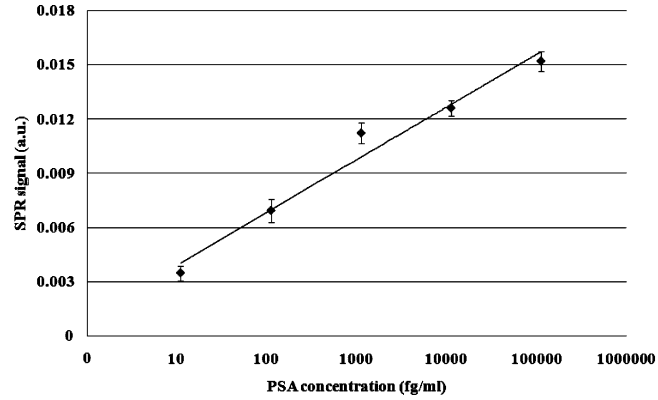

Figure 4. The linear relationship ($R^2 = 0.9724$) of t-PSA concentrations in PBS solution versus SPR signals. The error bar is equivalent to 1 standard derivation in this experiment.

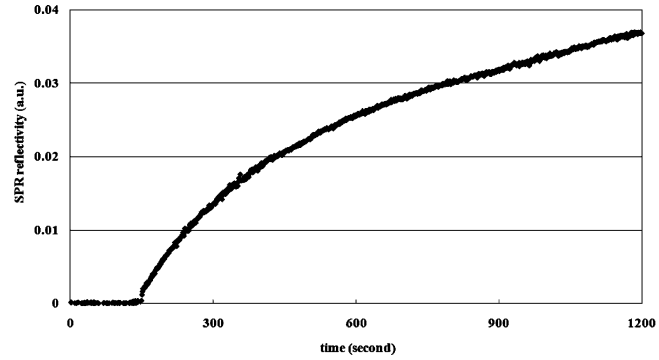

Figure 5. The binding processes of anti-t-PSA (capture antibody) interaction with t-PSA (61 pg/mL, target antigen) in 200-fold diluted human serum.

than the PSPWB. However, a limited dynamic range for the differential-phase SPR method was presented.

t-PSA Measurement in PBS or Diluted Human Serum. The CM5 chip was prepared using the method described previously. The covalent immobilization procedures of the anti-t-PSA to the dextran matrix on a CM5 chip were recorded by SPR reflectivity, as shown in Figure 3. The sensorgram describes the NHS/EDC activation (1), anti-t-PSA (39 μg/mL) immobilization (2), and deactivation by ETH (3). After the anti-t-PSA was immobilized onto the CM5 chip, the t-PSA in PBS solution or diluted human serum was injected over the sensor surface to be measured by the PSPWB system. Different concentrations of the t-PSA, i.e., 0 fg/mL, 10 fg/mL, 100 fg/mL, 1 pg/mL, and 10 pg/mL in 1 mL volume each, were prepared in PBS solution separately and then were injected into the reaction chamber to interact with the immobilized anti-t-PSA to form the ⟨anti-t-PSA/t-PSA⟩ complex on the CM5 chip surface. The PBS solution is treated as zero concentration and the signal level at zero concentration is defined as the background level. The SPR signal was then calculated by subtracting the background level from the saturated average of the detected signal over the last 50 data points in the experiment. Again, a clear linear relationship between the SPR signals and the different concentrations of t-PSA in PBS solution over the range of 10 fg/mL (~300 aM) to 100 pg/mL (~3 pM) is shown in Figure 4. The correlation coefficient (R^2) was 0.9724 in this experiment where the error bar was defined as 1 standard deviation. In this experiment, SNR $\geq$ 8 was calculated at different concentrations of t-PSA in PBS solution.

It has been demonstrated that for human serum at 100-fold dilution, the matrix already has no effect on response of the immunosensors.[46] Therefore, the t-PSA in 200-fold diluted human serum was directly measured by using the PSPWB, as shown in Figure 5. Experimentally, the t-PSA of 61 pg/mL (2 pM) in diluted human serum in 1 mL volume was injected into the reaction chamber to interact with the immobilized anti-t-PSA. The temporal response of SPR reflectivity indicates that t-PSA was also successfully detected at a quite low concentration (61 pg/mL) in diluted human serum by using PSPWB.

Recently, different groups reported advanced detection techniques wherein the sensitivity of PSA detection in PBS solution reached the femtogram per milliliter level. For instance, Choi and co-workers used scanning tunneling microscopy-based electrical detection combined with gold nanoparticles to measure PSA concentrations as low as 10 fg/mL (~300 aM).[47] The Rudbeck laboratory at Uppsala University developed new triple-binder proximity ligation assays for sensitive detection at as few as 300 protein complexes diluted in 5 μL of buffer.[48] Kim et al. utilized an n-type silicon nanowire-based structure field-effect transistor to improve the detection limit to 1 fg/mL (~30 aM).[49] Although the PSPWB is not sensitive as compared with those advanced detection techniques, its simplicity and applicability do have significant contributions in analysis of biorecognition or clinical diagnostics. In this study, the PSPWB verified that the detection limit can be 10 fg/mL (~300 aM) in real-time t-PSA detection without labeling. In addition, a wide dynamic range of 10^5 [10 fg/mL (~300 aM) to 100 pg/mL (~3 pM)] has been demonstrated as well. Most importantly, the PSPWB successfully detected t-PSA of 61 pg/mL (~2 pM) in diluted human serum experimentally. Consequently, the abilities of PSPWB on

(46) Suwansa-ard, S.; Kanatharana, P.; Asawatreratanakul, P.; Wongkittisuksa, B.; Limsakul, C.; Thavarungkul, P. *Biosens. Bioelectron.* **2009**, *24*, 3436–3441.

(47) Choi, J. W.; Oh, B. K.; Jang, Y. H.; Kang, D. Y. *Appl. Phys. Lett.* **2008**, *93*, 033110.

(48) Schallmeiner, E.; Oksanen, E.; Ericsson, O.; Spangberg, L.; Eriksson, S.; Stenman, U. H.; Pettersson, K.; Landegren, U. *Nat. Methods* **2007**, *4*, 135–137.

(49) Kim, A.; Ah, C. S.; Yu, H. Y.; Yang, J. H.; Baek, I. B.; Ahn, C. G.; Park, C. W.; Jun, M. S.; Lee, S. *Appl. Phys. Lett.* **2007**, *91*, 103901.

nonlabeling real time measurement and high detection sensitivity can be potentially applied to the clinical sample. Moreover, the PSPWB potentially shows both qualitative and quantitative measurement of protein−protein interactions with a high throughput chip-based assay that can be used for early diagnosis.[13,50]

CONCLUSIONS

The high-sensitivity performance of the PSPWB in detecting effective refractive index variation relies on the following properties. First, a highly spatial and temporal correlated two-frequency paired linear-polarized laser beam in a common-path interferometer is required. This makes the PSPWB capable of exciting a pair of highly correlated SPWs on a gold/dielectric medium interface simultaneously; thus, synchronized detection at a high SNR of heterodyne signals is produced.[25] Meanwhile, environmental disturbances and laser frequency fluctuations are prevented because of the common-phase noise rejection mode in the PSPWB. The effect of temperature variation in the reaction chamber of the SPR device is canceled out. However, the surface roughness of the gold thin film degrades the correlation between the paired SPWs on the interface. As a result, the detection sensitivity is lessened. High-quality gold thin film, therefore, is required in the PSPWB to ensure highly sensitive detection of biomolecular interactions in real time.

Second, the amplitude ratio algorithm was adopted in this experiment, and the excess noise from laser intensity fluctuations were reduced efficiently. This was critical for high detection sensitivity with the PSPWB. Consequently, the detection limit of the PSPWB at 10 fg/mL (∼300 aM) of t-PSA in PBS buffer was demonstrated and, to our knowledge, this is the highest detection sensitivity reported using SPR-based biosensors.[41] In addition, t-PSA of 61 pg/mL (∼2 pM) in diluted human serum was detected successfully.

In this work, a simple strategy using the PSPWB is demonstrated for high sensitivity and real-time detection of PSA without labeling. The detection limit of PSPWB in Δn_{eff} is 8.4×10^{-9} RIU (SNR = 3) corresponding to the measurement of 0.000 01 wt % sucrose−water solution. In PBS solution, the PSPWB biosensor achieves 10 fg/mL (∼300 aM) for t-PSA and a linear relation between the SPR signals and the concentrations of t-PSA was obtained in a range from 10 fg/mL (∼300 aM) to 100 pg/mL (∼3 pM). Therefore, the PSPWB can open up possibilities for the detection of either low molecular weight molecules or ultra low concentrations of macromolecule interactions without labeling for single molecular detection consideration. Furthermore, t-PSA of 61 pg/mL (∼2 pM) in diluted human serum was successfully detected. This shows the advantage of having the capability of detecting target analytes in clinical sample without complicated operating procedures.

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(50) Wong, C. L.; Ho, H.-P.; Suen, Y. K.; Kong, S. K.; Chen, Q. L.; Yuan, W.; Wu, S. Y. *Biosens. Bioelectron.* **2008**, *24*, 606–612.