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A novel surface plasmon resonance multisensing separated by multichannels for biosensor applications

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ABSTRACT

A novel surface plasmon resonance (SPR) multisensing, which does not require imaging apparatus, has been proposed. The SPR signal from the different ligands can be separated by the control of the thicknesses of multichannel layers, which enhances the measurement resolution. The proposed method is verified by the calculations based on Fresnel reflection model.

Key words : surface plasmon resonance, multisensing, multichannel, imaging

Introduction

Asurface plasmon resonance (SPR) based sensors have been widely applied for various measurements in the field of biology, chemistry and thin films during the last decade (1-4). Especially, the success of SPR technique in biological applications is due to three factors: real-time measurement of biomolecular interactions on the sensor surface and no labeling or no marker for detection of biomolecules and high resolution along the thickness of analyte (1).

In modern biotechnology, microarray-based assays are becoming increasingly important, which requires an accurate sensing system to detect various signals from arrayed ligands simultaneously (5). Therefore, a new strong demand on SPR measurements in biotechnological applications is sensing on several areas of the sensor surface at the same time. This is called SPR multisensing. Several researchers have reported their work in SPR multisensing. Guedon et. al. (6) used six kinds of nucleotide with different thickness and probe density as arrayed ligands. They used the 8-bit CCD camera to measure the arrayed ligands and reported measured results only for each ligand not for the arrayed ligands measured simultaneously. Berger et. al. (7) measured 4x4 different kinds of antibody-antigen reactions by recording them using CCD video camera under the condition of the fixed wavelength and incident angle, which means different reaction sensitivity for different ligands because the reactions of ligands are different each other and vary depending on the incident angle and the wavelength. Lee et. al. (8) measured the SPR difference images for 3x2 arrays of different DNA samples. They used 4x magnification objective lens, CCD camera and fluorescencemicroscopy for image measurements. They obtained the difference of images for the arrayed ligands measured under the condition of the fixed wavelength and incident angle. Some other researchers (9-11) have reported similar results with above three cases.

These results of SPR multisensing have the common features: (1) they are measured by using CCD camera and relevant software for data processing (2) image for arrayed ligands can be obtained only when the incident angle and wavelength are fixed simultaneously; (3) when the incident

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angle or wavelength is changed within certain range, only the image for the each ligand (not for the arrayed ligands) can be measured sequentially. In view of arrayed assay, where the reaction of the different ligand should be monitored simultaneously, the above features (2) and (3) may be a serious problem. Also, the features (2) and (3) are due to the use of imaging apparatus such as CCD camera.

In this paper, we present a novel SPR multisensing, which uses the regular SPR measurement set-up, so the SPR signal is detected by the spectrometer or the photodetector not imaging apparatus such as CCD camera. The proposed method is based on the multichannel SPR (12-16) and the separation of signals by use of multichannel layers with different thicknesses. Fresnel reflection model (17) is used for verification of the proposed method.

New SPR multisensing based on multichannel

Application of multichannel to SPR multisensing

The problems of the current multisensing are caused by the using of imaging apparatus such as a CCD camera. The method to observe the effect of refractive index change for each ligand in real time and at the same time without imaging devices, which implies the using of the regular SPR, is essential in biological applications. Multichannel SPR can afford a basis for this kind of effective two dimensional multisensing.

Multichannel SPR initially proposed by Yee group (12-14) and Karube group (15, 16) has the purpose of compensating non-specific reaction on the sensor surface. As shown in **Fig 1(a)**, multichannel SPR has two different channels, one is reference channel and the other is sensing channel. The purpose of the reference channel is to discriminate the specific response from refractive index variations due to background interference and non-specific response. The reference channels respond only to non-specific effects. The expected result of multichannel SPR is shown in **Fig 1(b)**. The reflectance of the multichannel SPR is the summation of each reflectance from the reference channel and the sensing channel considering the ratio of the illuminated region area (14).

If we compose the multichannel SPR by different ligands not by the reference channel and the sensing channel, it can be used as an SPR multisensing. This novel concept is shown in **Fig 2(a)**, where three different kinds of ligands are illuminated and detected by wavelength modulation. Because each different ligand has different refractive index, the SPR wavelengths of three ligands are different. Also, since the reaction in three different ligands is different, the wavelength shift in three ligands is different. The expected result shown in **Fig 2(b)** is the result from the regular SPR device in wavelength modulation and it can show the refractive index change in different ligands in real time and at the same time. In **Fig 2(b)**, the SPR wavelengths for three different ligands are λ_1, λ_2 and λ_3, respectively. λ_0 corresponds to the SPR wavelength of the Au substrate. After the reaction, the refractive index changes are different in three

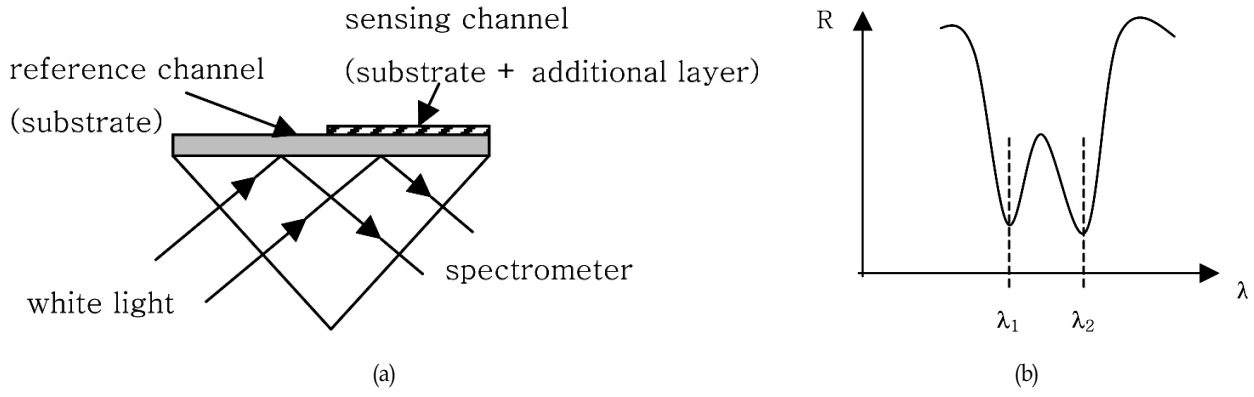

Fig 1. (a) Multichannel SPR is composed of reference channel and the sensing channel, (b) there are two SPR wavelengths, λ1 is for reference channel and λ2 is for sensing channel.

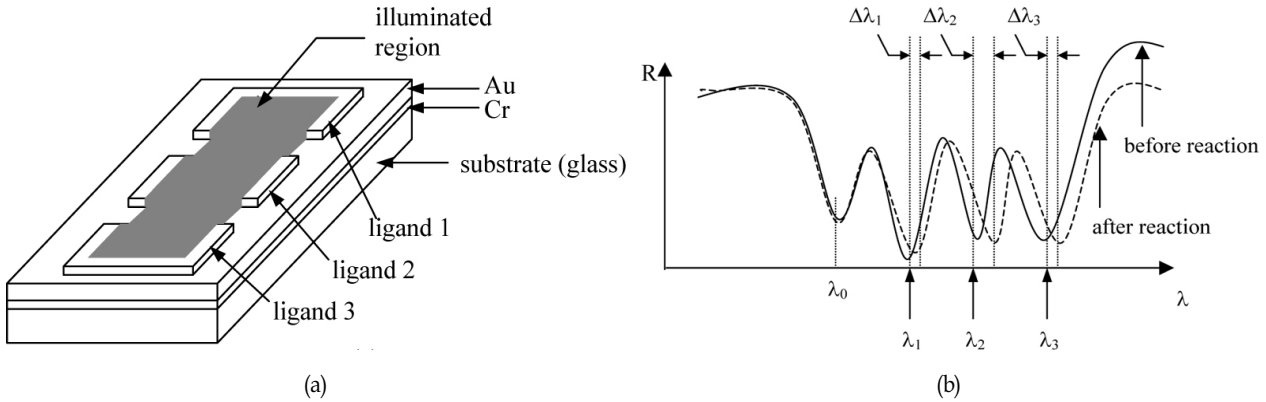

Fig 2. SPR multisensing using multichannel, (a) different ligands are patterned on the substrate and illuminated. Reflection is measured using the regular wavelength modulation SPR, (b) the SPR wavelengths before reaction are 0, 1, 2 and 3, for substrate, ligand 1, ligand 2 and ligand 3, respectively. After the reaction, SPR wavelengths of three ligands are shifted according to their refractive index variations.

ligands and they are denoted by the SPR wavelength shift, $\Delta\lambda_1$, $\Delta\lambda_2$ and $\Delta\lambda_3$, respectively. For the SPR wavelength of the substrate, there is no change in SPR wavelength before and after the reaction because the refractive index variation stems from the ligand-analyte reaction.

Novel SPR multisensing based on the separation of signals by multichannel

Multichannel SPR, of which channels are composed of different ligands, can be used for SPR multisensing as shown in **Fig 2.** It shows the SPR wavelength shift due to the refractive index variation at each ligand independently and simultaneously. In order to observe the SPR signals and variation from each ligand clearly, it is necessary that each reflection curves for ligands should be separated enough to discriminate each responses. Unfortunately, this is not possible in the configuration shown in **Fig 2(a)**. When the refractive index variation is measured by the regular SPR device, the SPR curves for different refractive index are positioned very closely. Therefore, if all the ligands are illuminated simultaneously, the SPR curves from each ligand are overlapped and it is very difficult to discriminate each curve.

This problem can be resolved by the structure shown in **Fig 3**, in which the SPR signals from each ligand are separated by multichannel. The multichannel in the structure of **Fig 3** has additional layer with different thickness, on which each ligand is patterned. For example, in **Fig 3**, the additional layer is three different Ta_2O_5 layers with thickness of 10 nm, 20 nm and 30 nm, respectively if t_1, t_2 and t_3 are 10 nm. Three different ligands are positioned on three different additional layer (Ta_2O_5). The SPR wavelength (in wavelength modulation) or SPR angle (in angle modulation) is seriously dependent on the thickness of the layer on the substrate and controllable by the variation of thickness (18). The precise control of the additional layer, usually oxide layer, is easily possible, while the thickness of the ligand is limited by preparation procedure. Therefore, the SPR signals from each

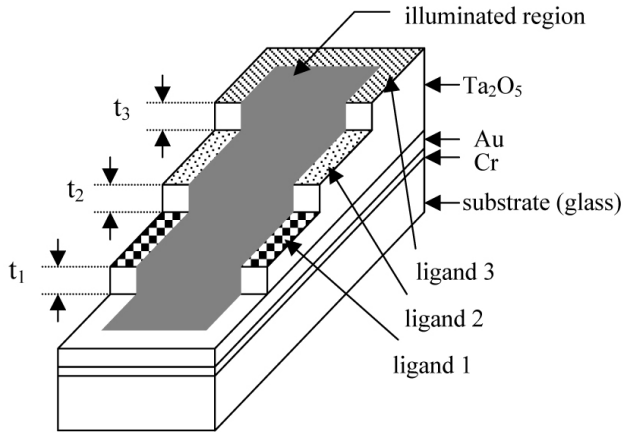

Fig 3. The structure of the novel SPR multisensing separated by multichannel. Three channels are Ta_2O_5 layers of which thicknesses are t1, t_1+t_2 and $t_1+t_2+t_3$, respectively. Three different ligands are patterned on each channel, so the SPR signals from each ligand can be separated by multichannel with different thickness.

ligandcan be separated enough to be discriminated by the control of the thickness of the inserted additional layer in multichannel. The multichannel structure can be designed in various shapes according to the arrayed patterns of ligands. A step-like structure shown in **Fig 3**, which is similar structure with previously reported resonant mirror (19), is a just simple example to implement the proposed methodand various structure design is possible besides one shown in **Fig 3**.

Calculated results

Modeling

The usefulness of the proposed SPR multisensing has been proved by the calculation based on the Fresnel reflection model (17).

Assuming the SPR device composed of N layers from the prism to the ligand, the reflection coefficient Γ can be expressed as follows.

$$\Gamma = \frac{A + \frac{B}{R_N} - R_1(C + \frac{D}{R_N})}{A + \frac{B}{R_N} - R_1(C + \frac{D}{R_N})} \tag{1}$$

where

$$\begin{bmatrix} A & B \\ C & D \end{bmatrix} = \prod_{m-2}^{N-1} \begin{bmatrix} A_m & B_m \\ C_m & D_m \end{bmatrix} \tag{2}$$

$$= \prod_{m=2}^{N-1} \begin{bmatrix} \cos(k_{mz} t_m) & j r_m \sin(k_{mz} t_m) \\ j \frac{\sin(k_{mz} t_m}{R_m} & \cos(k_{mz} t_m) \end{bmatrix}$$

and where t_m and n_m are the thickness and refractive index of mth layer respectively.

Other parameters in Eq. (2) can be written as

$$R_m = \frac{k_{mz}}{\omega \epsilon_m} = \frac{k_{mz} \lambda}{2\pi c \epsilon_o n_m^2} \tag{3}$$

$$k_{mz} = k_m \cos\theta_m = k_0 n_m \cos\theta_m \tag{4}$$
$$= k_0 n_m [1 - (\frac{n_1}{n_m})^2 \sin^2\theta_1]^{\frac{1}{2}}$$

where k_0 is $2\pi/\lambda$. Given the thickness and refractive index of each layer, the incident angle and the wavelength, the reflection coefficient can be calculated using Eqs. (1)-(4). The reflectance is the square of the reflection coefficient, so we can get the SPR curve varying the incident angle or the wavelength.

Results and Discussion

Calculated results using Fresnel reflection model for the case of **Fig 2(a)**, in which different three ligands are patterned on the Au substrate, are shown in **Fig 4**. Refractive indexes of the three ligands are assumed as 1.40, 1.55 and 1.70, respectively. The SPR measurement is done by the wavelength modulation and the incident angle is 70°. **Fig 4(a)** shows each SPR curve for three different ligands. The curve of which SPR wavelength is about 722 nm corresponds to ligand 1 of which refractive index is 1.40. Also, the SPR wavelength of 748 nm is for the ligand 2 (refractive index is 1.55) and 771 nm is for the ligand 3 (refractive index is 1.70). The curve with the SPR wavelength of 710 nm is for the substrate. We assumed the substrate as layers of Au of 50 nm thickness, Cr of 2 nm and the glass. The refractive index of 1.334 for the substrate is the value for water on the surface of the substrate. **Fig 4(a)** is the result when the SPR measurements are performed for each ligand independently. If we measure by the simultaneous illumination to the three ligands as shown in **Fig 2(a)**, the resultant SPR curve is the summation of three curves for each ligand considering the area, which is shown in **Fig 4(b)** as a solid line. Four curves in **Fig 4(a)** are merged together and it is impossible to discriminate each curve. The case is the same after the reaction on the sensor surface as shown in **Fig 4(b)** as a dotted line. This result means that the sensor configuration in **Fig 2(a)** can not be used for the SPR multisensing which can measure the SPR signals from different ligands in real time and at the same time.

Fig 5 shows the result when the SPR signals are separated by the additional layers with different thickness. As shown in **Fig 4**, there are three different channels, which are Ta_2O_5 with the thickness of t_1 (10 nm), t_1+t_2 (20 nm) and $t_1+t_2+t_3$ (30 nm), respectively. Three different ligands are pat-

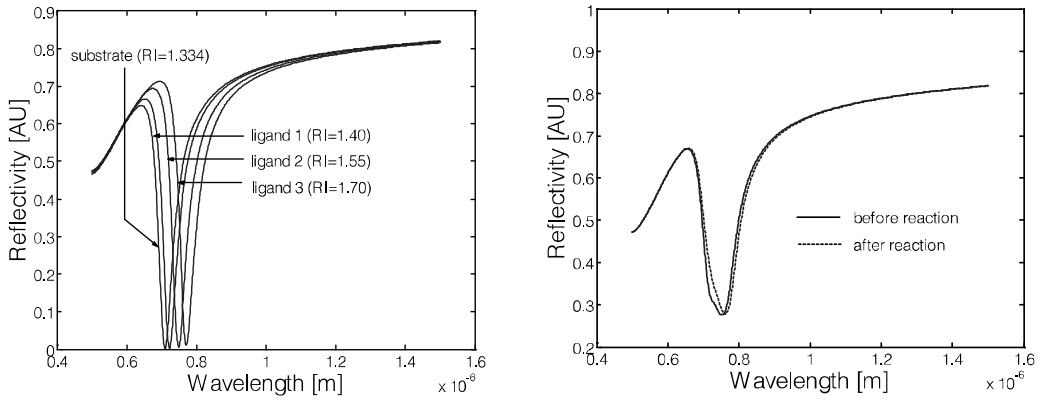

Fig 4. Calculated results for the SPR measurement with the configuration shown in **Fig 2** (a); (a) three different ligands are measured independently and they are positioned very closely. RI means refractive index, (b) three different ligands are illuminated simultaneously and measured, but all the curves are merged together and it is impossible to discriminate.

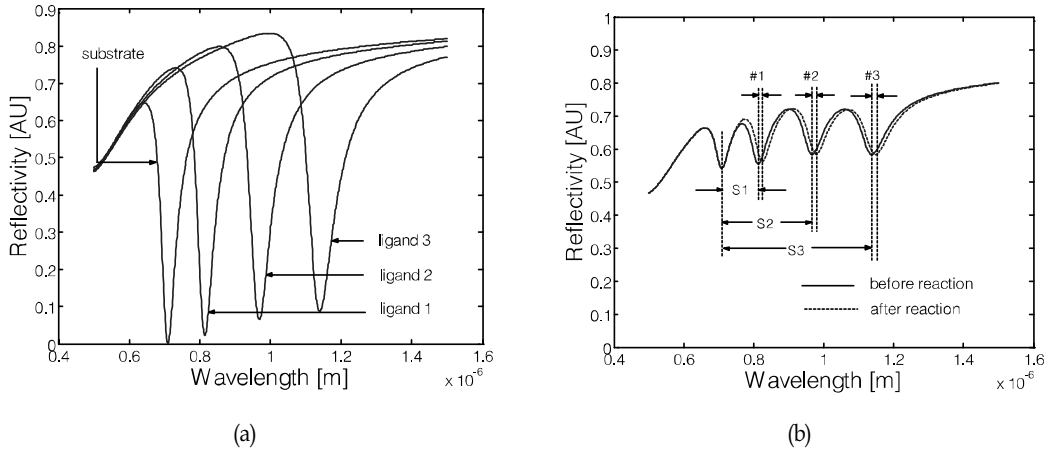

Fig 5. Calculated results for the SPR measurement with the configuration shown in **Fig 3**; (a) three different ligands are measured independently and they are separated apparently by the additional layers with different thickness. Refractive indexes for different ligands are same in **Fig 4**, (b) three different ligands are illuminated simultaneously and measured, and all the curves are separated before and after the reaction and they are obvious to discriminate. S_1, S_2 and S_3 denote the resonance separation between the SPR wavelength for Au surface and for each ligand. Also, #1, #2 and #3 imply the resonance shift due to the refractive index change in each ligand.

terned on each channel, so ligand 1 is on the Ta_2O_5 layer of 10 nm, ligand 2 on 20 nm and ligand 3 on 30 nm. The result when each ligand is measured independently is shown in **Fig 5(a)**. The curve of which SPR wavelength is about 814 nm corresponds to ligand 1 on the Ta_2O_5 layer of 10 nm. Also, the SPR wavelength of 968 nm is for the ligand 2 on 20 nm and 1140 nm is for the ligand 3 on 30 nm. The refractive index of each ligand is assumed to be the same in **Fig 4.** The curve with the SPR wavelength of 710 nm is for the substrate. Comparing with the result in **Fig 4(a)**, it is obvious each signal from different ligands is apparently separated. This is because Ta_2O_5 layers with different thickness separated the SPR wavelengths proportional to their thickness. When all the ligands are illuminated simultaneously as shown in **Fig 3**, four curves in **Fig 5(a)** are merged. Since the SPR signals from each ligand are sepa-

Table 1. Used Parameters in Calculatio

	Ligand w/o Ta_2O_5		Ligand w/ Ta_2O_5	
	before reaction	after reaction	before reaction	after reaction
Configuration	**Fig 2**(a)		**Fig 3**	
Substrate	Au (50nm) / Cr (2 nm) / glass (0.5 mm)		Au (50 nm) / Cr (2 nm) / glass (0.5 mm)	
Channel				
1st	ligand 1 (10 nm)		ligand 1 (10 nm) / Ta_2O_5 (10 nm)	
2nd	ligand 2 (10 nm)		ligand 2 (10 nm) / Ta_2O_5 (20 nm)	
3rd	ligand 3 (10 nm)		ligand 3 (10 nm) / Ta_2O_5 (30 nm)	
Refractive index				
ligand 1	1.40	1.45	1.40	1.45
ligand 2	1.55	1.60	1.55	1.60
ligand 3	1.70	1.75	1.70	1.75
Calculated results	**Fig 4**(b) solid line	**Fig 4**(b) dotted line	**Fig 5**(b) solid line	**Fig 5**(b) dotted line

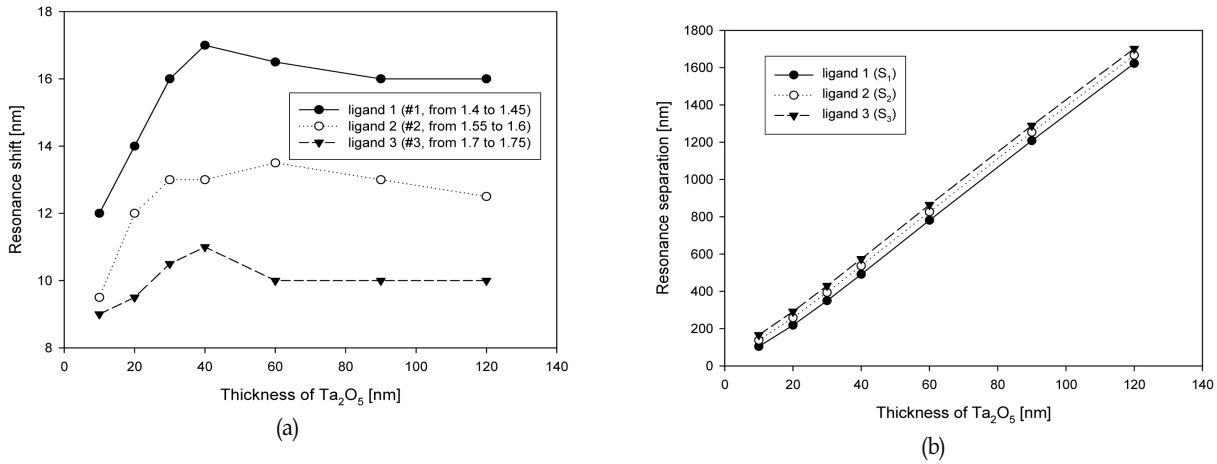

Fig 6. Calculated results for (a) the resonance shift and (b) the resonance separation. The resonance shift shows some trend of maximum or almost saturation at around 40 - 80 nm. The resonance separation shows a simple linear relation with the thickness of Ta_2O_5

rated, the merged signal has four separated SPR signals, which is shown in **Fig 5(b)** as a solid line. Assuming the refractive index of each ligand is changed from 1.40 to 1.45 in ligand 1, from 1.55 to1. 60 in ligand 2 and from 1.70 to 1.75 in ligand 3 by the reaction on the sensor surface, the solid line in **Fig 5(b)** is changed to the dotted line. After the reaction, the SPR signals from different ligands are still separated and we can measure the shift of the SPR wavelength. This result means that the proposed SPR measurement configuration as shown in **Fig 3**, can be used for the SPR multisensing which enables the real time and simultaneous measurements for different ligands array. Used parameters and values are summarized in Table 1.

In the calculation of **Fig 5,** the thicknesses of the additional layers are assumed to be 10, 20 and 30 nm. The variation of the thickness of the additional layer results in the change of the resonance shift and the resonance separation. The resonance shift means the change of SPR wavelength before and after reaction, which is indicated as #1 (in ligand 1), #2 (in ligand 2) and #3 (in ligand 3) in **Fig 5(b)**. The resonance separation is the separation between the SPR wavelength for Au surface and for each ligand, which is shown as S_1, S_2 and S_3 in **Fig 5(b)**. **Fig 6** shows the calculated resonance shift and resonance separation for various thicknesses of the additional layer. The result for the resonance shift in **Fig 6(a)** shows some trend of maximum or almost saturation at around 40-80nm, while the resonance separation in **Fig 6(b)** shows a simple linear relation with the thickness of Ta_2O_5. The larger resonance shift is desirable in the point of the device sensitivity and the resonance separation should

be enough to discriminate each SPR signals from different ligands. **Fig 5(b)**, which is the result for Ta_2O_5 thicknesses of 10, 20 and 30 nm, shows the thickness of 10 nm as a difference of the additional layer for different ligands is enough to separate each signals apparently. Therefore the optimum thickness of the additional layer can be decided to be about 40-80 nm considering the range of the maximum resonance shift.

Conclusion

A novel SPR multisensing, which does not require imaging apparatus and can be implemented using the regular SPR technique, has been proposed. The SPR signals from the different ligands are separated by the control of the thickness of the additional layers in multichannel, which enhances the measurement resolution. The proposed method has been verified by the calculations based on Fresnel reflection model. The proposed method, as evidenced by our calculated results, can be used for measurements of SPR signals from different ligand array in real time and at the same time, which can be a useful tool in biological technologies.

References

(1) Silin V, Plant A (1997) Tibtech **15**, 353
(2) Homola J, Yee SS, Gauglitz G (1993) Sensors and Actuators B **54**, 3
(3) Liedberg B, Nylander C, Lundstrom I (1995) Biosensors and Bioelectronics **10**, i
(4) Johnston KS, Karlsen S, Jung CC, Yee SS (1995) Materials Chemistry and Physics **42**, 242
(5) Joos TO, Stoll D, TemplinMF (2001) Current Opinion in Chemical Biology **6**, 76
(6) Guedon P, Livache T, Martin F, Lesbre F, Roget A, Bidan G, Levy Y (2000) Analytical Chemistry **72**, 2003
(7) Berger CE, Beumer TAM, Kooyman RPH, Greve J (1998) Analytical Chemistry **70**, 703
(8) Lee HJ, Goodrich TT, Corn RM (2001) Analytical Chemistry **73**, 5525
(9) O'Brien MJ, Perez-Luna VH, Brueck SRJ, Lopez GP (2001) Biosensors and Bioelectronics **16**, 97
(10) Xinglong Y, Dongsheng W, Dingxin W, Hua OJ, Zibo Y, Yonggui D, Wei L, Xin Z (2003) Sensors and Actuators B **91**, 133
(11) Palumbo M, Pearson C, Nagel J, Petty MC (2003) Sensors and Actuators B **90**, 264
(12) Nenninger GG, Clendenning JB, Furlong CE, Yee SS (1998) Sensors and Actuators B **51**, 38.
(13) Homola J, Lu HB, Yee SS (1999) Electronics Letters **35**, 1105
(14) Homola J, Lu HB, Nenninger GG, Dostalek J, Yee SS (2001) Sensors and Actuators B **76**, 403
(15) Akimoto T, Sasaki S, Ikebukuro K, Karube I (2000) Analytica Chimica Acta **417**, 125
(16) Akimoto T, Ikebukuro K, Karube I (2003) Biosensors and Bioelectronics **18**, 1447
(17) Taya M (2004) "Electronic Composites" Cambridge University Press, New York.
(18) Johnston KS (1995) "Characterization of Thin Films Using Surface Plasmon Resonance" Master Thesis, University of Washington, Seattle.
(19) Cush R, Cronin JM, Stewart WJ, Maule CH, Molley J, Goddard NJ (1993) Biosensors and Bioelectronics **8**, 347

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