Prediction of the limit of detection of an optical resonant reflection biosensor

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Abstract: A prediction of the limit of detection of an optical resonant reflection biosensor is presented. An optical resonant reflection biosensor using a guided-mode resonance filter is one of the most promising label-free optical immunosensors due to a sharp reflectance peak and a high sensitivity to the changes of optical path length. We have simulated this type of biosensor using rigorous coupled wave theory to calculate the limit of detection of the thickness of the target protein layer. Theoretically, our biosensor has an estimated ability to detect thickness change approximately the size of typical antigen proteins. We have also investigated the effects of the absorption and divergence of the incident light on the detection ability of the biosensor.

OCIS codes: (230.1950) Diffraction gratings; (230.7400) Waveguides, slab.

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1. Introduction

Biosensors that can detect the presence of certain kinds of biomolecules are important ingredients for biomedical applications. The target material for a biosensor can be any kind of biomolecular complex, such as DNAs, proteins and cells. Some of these materials can act as an antigen to produce an antibody:anitgen complex, which is known as an immunocomplex, by reacting with the corresponding antibody. These immunological reactions can be adopted as the main principle for the capture of the target materials in a sample.

After capturing the target materials, the detection mechanism is needed to convert the molecular binding to a signal that can be measured. One method for doing this is to label the resulting complex with compounds such as fluorescent, radioactive, or colorimetric. However, this method can increase the test complexity and alter the functionality of the molecules caursing errors. To overcome these disadvantages, label-free methods have been developed using the surface acoustic wave [1], field effect transister [2], porous silicon [3], or optical techniques such as total internal reflection ellipsometry [4, 5], surface plasmon resonance [6], or interferometry [7]. Typically, these label-free biosensors have high sensitivity, high signal to noise ratio and an ability to monitor target materials in real time.

Guided mode resonance [8] is one of the optical techniques that can be used as a transducing method in biosensing. An optical resonant reflection biosensor, using the guided mode resonance filter(GMRF), can detect the changes in thickness or refractive index of the biomolecular layer absorbed on the filter surface by monitoring shift of sharp resonance peak in the spectrum of the light reflected (or transmitted) from the GMRF [10]. A GMRF is a diffraction grating with a subwavelength periodic structure made of high refractive index material, which enables the grating to guide the incident light. We can calculate the reflection spectrum from the GMRF by applying the rigorous coupled wave theory [11] to the optical structure. The resulting sharp resonance and the high sensitivity on the optical thickness of the adjacent layers of the GMRF allows use of the highly sensitive optical biosensor.

In this work, we have estimated the limit of detection by calculating the shift of the resonant reflection peak due to the changes in the thickness of the bio-material layer on the upper surface of the optical resonant reflection biosensor.

2. Modeling optical resonant reflection biosensor

A single period of the GMRF with the resonance wavelength around 780 *nm* for biosensing is presented in Fig. 1. It is comprised of a glass substrate (n_{sub}=1.47), a grating made of polymer resin (n_{resin}=1.55) on the substrate, and high refractive index coating (n_{high}=2.00) with materials such as SiN_x or TiO_2 on the grating. The physical dimension of the GMRF is shown in Fig. 1(b) Antibodies were immobilized to capture the target antigens on the top surface of the GMRF. To calculate the reflection spectrum, it was necessary to model the grating structure as a stack of layers with a periodically modulated refractive index. Suppose that the z-axis is along the direction from the substrate to the superstrate and the x-axis is from left to right in Fig. 1, the electric field of the guiding mode in the ith layer satisfies the wave equation : [8, 11]

$$E_{y,i} = \sum_{n=-\infty}^{+\infty} \hat{S}_{n,i}(z) \exp(\mathbf{j}nKx). \tag{1}$$

Where the normal incidence is assumed from the $+z$ direction, the polarization direction is y, K is the wavevector of the refractive index modulation, and $\hat{S}_{n,i}$ satisfies the differential equation :

$$\frac{d^2\hat{S}_{n,i}}{dz^2} + (k^2\varepsilon_g - n^2K^2)\hat{S}_{n,i} + k^2 \sum_{m=-\infty}^{+\infty} \varepsilon_{m,i}\hat{S}_{n-m,i} = 0. \tag{2}$$

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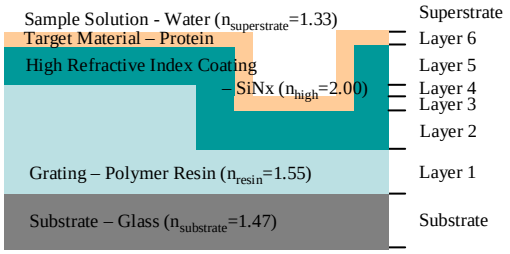
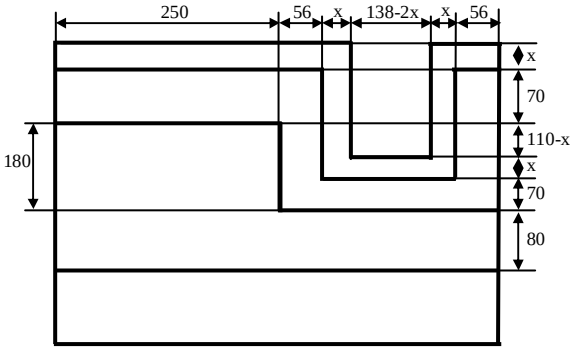


(a)



(b)

Fig. 1. (a) The layered structure of a single period of our guided mode resonance filter (b) The physical dimensions are in *nm* unit for the guided mode resonance filter.

Here, it is assumed that the permittivity of the ith layer $\varepsilon_i(x) = \varepsilon_g + \sum \varepsilon_{m,i} e^{\mathbf{j}mKx}$. The electric field in the substrate and superstrate area will be the wave propagating into the free space.

$$E_{y,sup} = \exp(-\mathbf{j}n_{sup}kz) + \sum_n R_n \exp\left(\mathbf{j}nKx + \mathbf{j}\sqrt{\varepsilon_{sup}k^2 - n^2K^2}z\right) \tag{3}$$

$$E_{y,sub} = \sum_n T_n \exp\left(\mathbf{j}nKx - \mathbf{j}\sqrt{\varepsilon_{sub}k^2 - n^2K^2}(z-d)\right) \tag{4}$$

where ε_{sub} and ε_{sup} are the permittivity of the substrate and superstrate, respectively. The reflectance and transmittance of the whole filter can be found by solving the boundary conditions between every layeral interfaces after converting the Eq. 2 into an eigensystem problem.

3. The limit of detection for the thickness of the target protein layer

The sensitivity of the biosensor can be estimated by evaluating the limit of detection which is the least amount of the target material that will produce a detectable signal. For the optical resonant reflection biosensor, detected physical quantity is the thickness of the target protein layer on the surface of the GMRF, and the signal is the shift of the peak in the reflection spectrum. Therefore, we can define the limit of detection of our biosensor as a ratio of least measureable peak shift and the rate of the peak shift during the thickness change.

$$LOD = \frac{\Delta\lambda_{detectable}}{\left(\frac{\Delta\lambda_{spectrum}}{\Delta t_{protein}}\right)} = \frac{10\% \times FWHM}{\left(\frac{\Delta\lambda_{spectrum}}{\Delta t_{protein}}\right)} \tag{5}$$

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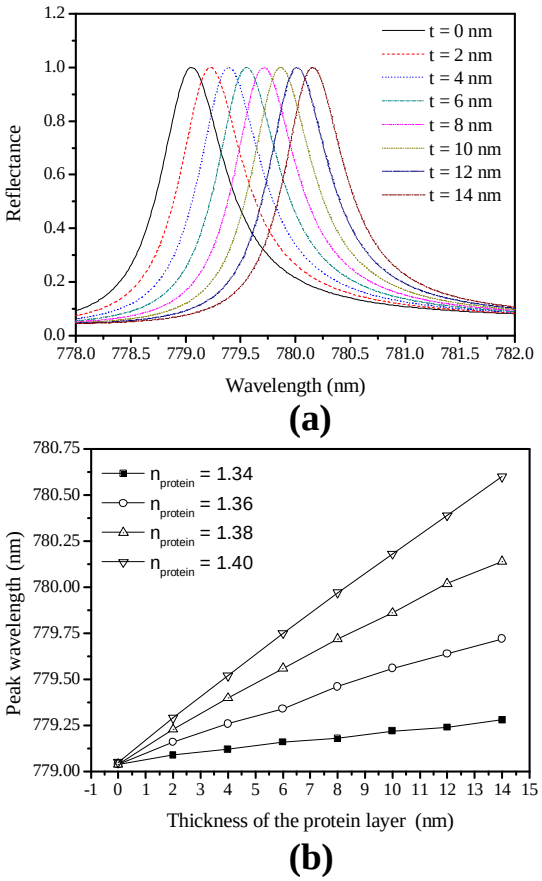

Fig. 2. (a) The shift of the reflection spectrum from the guided mode resonance filter due to the thickness changes of the protein layer when the refractive index of the layer is 1.38. (b) The amounts of the spectrum shift with different refractive index of the protein layer

Here it is assumed that a wavelength shift of 10% of the full width half maximum (FWHM) of the resonance peak can be detected. This assumption is valid while the FWHM of the reflection spectrum of our grating is 10 times wider than the resolution of the spectrometer used to measure the reflection spectrum. The calculated FWHM of the grating, about 0.7 *nm*, fulfills the standard for commercially available spectrometers, which have resolution approximately 0.1 *nm*. This standard can be easily met, when a tunable laserdiode like VCSEL is used with a photodetector [9].

The responses of the GMRF to the thickness changes in the target protein layer are found by caculating the reflection spectrum of the structure in Fig. 1 with different thickness *x*. The resultant response when the refractive index of the protein layer is 1.38 is shown in Fig. 2(a) with the asumption that the refractive index of the superstrate (a buffer solution that is assumed to be water) is about 1.33. The reflection spectrum shifts about 0.74 *nm*, with protein layer thickness changes of about 10 *nm*. can be concluded that proteins of 1 *nm* size can be detected if the refractive index is 1.38.

As shown in table 1, the estimated limit of detection for the thickness of the protein layer, that is approximately the size of the protein molecule, depends on the refractive index of the target protein itself. In the worst case, the limit is about 5 *nm*, which is approximately equal to the

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typical size of the proteins with a mass of a few tens of Da. The smallest size of a target protein molecule should also diminish as the difference between the refractive index of the protein and that of the buffer solution diminish. From this point, the sensitivity of the optical biosensor can be improved by using buffer solutions with a lower refractive index as the typical refractive index of the proteins is greater than that of buffers.

Table 1. The limit of detection of our optical resonant reflection biosensor on the size of the target protein.

the refractive index of the target protein	limit of detection on the size
1.34	5.00 *nm*
1.36	1.60 *nm*
1.38	1.00 *nm*
1.40	0.68 *nm*

4. Effect of absorptions and thickness of the high refractive index coating on the sensitivity

As our definition of the limit of detection in Eq. 5 depends on the FWHM of the reflection spectrum peak, the estimated limit of detection can be affected by the absorptions or scattering losses of the filter. We can simulate these effects by introducing imaginary refractive indices to materials that constitute the GMRF. Generally, absorption by the glass substrate is negligible compared to the polymer resin and the high refractive material used as coating. So it is sufficient to introduce an imaginary refractive index only to the resin and high refractive coating.

The simulated reflection spectrum from the filter with absorption in the resin and high refractive coating layer is shown in Fig. 3(a). The changes of the centeral wavelength of the resonance peak is negligible for the absorption. The FWHM dependence on the absorption is also shown in Fig. 3(b). The imaginary refractive index of 10^{-3} in resin and high refractive coating leads to a doubling of the the FWHM of the filter. This doubles the limit of detection.

The thickness of the high refractive coating also occurs to the FWHM of the filter. The estimated effects are shown in Fig. 4. The FWHM of the filter reaches its minimum at a thickness of 130 *nm*. We can optimize the sensitivity of the optical resonant reflection biosensor by controlling the thickness of the high refractive index material.

5. The influence of a beam divergency

As noted above, previous calculations have been done in the assumption of normal incidence. However, the reflection spectrum of the GMRF depends on the incident angle of light beam. Small error in the incident angle can cause shift of the peak wavelength in the reflection spectrum. This peak shift can also be calculated directly in the framework of the rigorous coupled wave theory. The calculated peak wavelength shift concerning the influence of the incident angle erroneously deviated from normal incidence in the plane perpendicular to the grating is shown in Fig. 5. All parameters used in the calculation are shown in Fig. 1 except for the target material layer. As shown in Fig. 5, the shift of the peak wavelength value is proportional to square of the angle of incidence in small incident angle regime. However, this shift due to the error in incident angle does not affect the limit of detection for our biosensor in the case of plain incident wave because the protein layer would introduce additional spectrum shift corresponding its refractive index and thickness.

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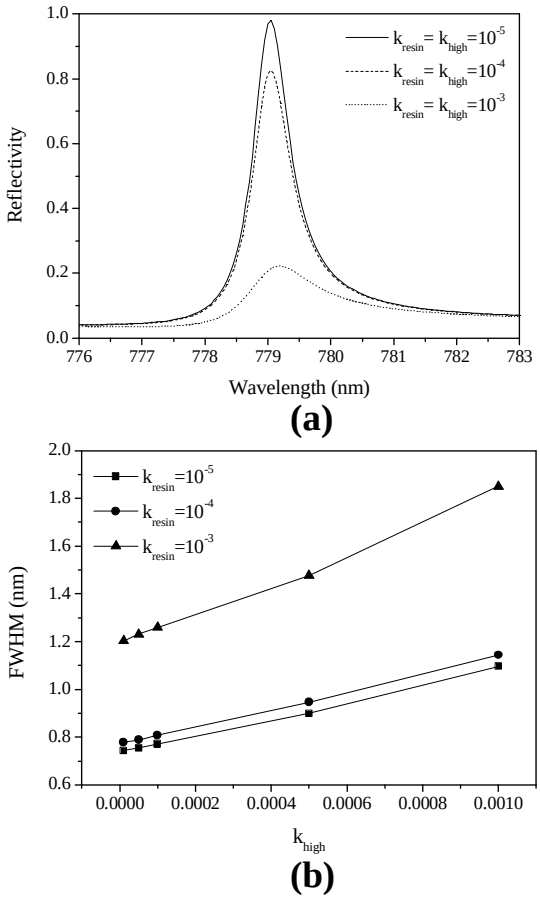

Fig. 3. (a) The effect of the absorption in the guided mode resonance filter. k_{resin} and k_{SiNx} are the imaginary refractive index of the resin and high refractive index material, respectively. (b) The FWHM effected by the absorption of the grating.

In a realistic situation, the incident beam should be considered to have divergency. This divergency introduces a distribution of the angle of incidence giving rise to the spread of reflection peak. We can estimate the effect of the beam divergency to the limit of detection relying on the FWHM of the peak from Fig. 5. For a Gaussian beam, the half angular divergence is given by

$$\theta \simeq \frac{\lambda}{\pi w_0} \tag{6}$$

where θ is in radians, λ is the wavelength of the incident light, and w_0 is the minimum spot size (i.e. spot size at the beam waist). When we use 780 *nm* as wavelength and 0.5 *mm* as the minimum spot size, the divergence will be 0.5 *mrad* (0.028°) that corresponds to 0.003 *nm* wavelength shift resulting in 0.8 % broadening of the reflection peak. For the commercially available lasers, typical divergence angle is from 0.5 *mrad* to 1.5 *mrad*. In the case of 1.5 *mrad*, the effect of the divergency is 0.03 *nm* shift corresponding to 8 % of FWHM broadening and 8 % of degradation of the detection ability. Therefore, most of target bio-materials will be still in the detectable range of our biosensor.

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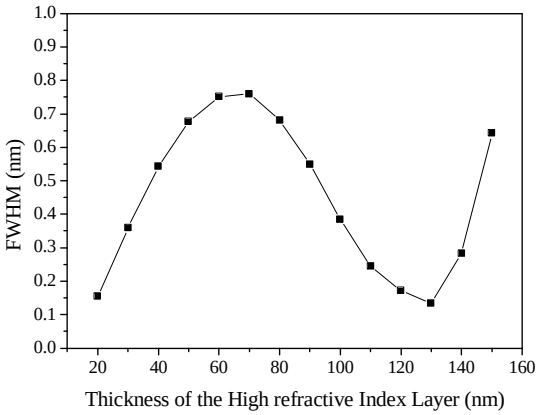

Fig. 4. The effect of the thickness of high refractive coating on the FWHM of the guided mode resonance filter.

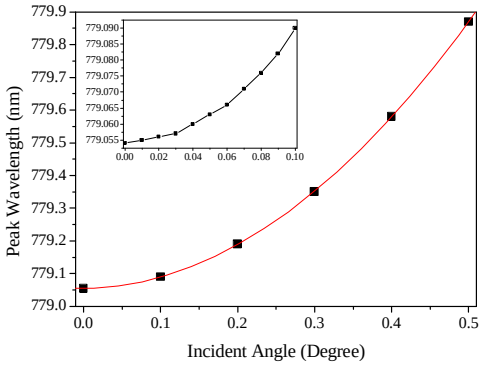

Fig. 5. The effect of angle of incidence on the reflection spectrum of the bare GMRF (without protein layer). The line is a 2nd order polynomial fitting curve to the peak value points.

6. Conclusion

In this work, we have presented the estimated limit of detection for our optical biosensor by simulating the reflection spectrum of the GMRF. The minimum size of the detectable protein molecule depends on the difference of the refractive index between the buffer solution and the target protein itself. Our modeled biosensor could detect down to several *nm* sized thicknesses the smallest of which was similar to the typical size of protein molecules with a few tens Da mass. The FWHM of the reflection peak, consequently the sensitivity of the optical biosensor, can be affected by the absorption and the thickness of the high refractive coating. With a 10^{-3} imaginary refractive index, the sensitivity would be half the non-absorptive case. The divergence of incident light wave also influences the FWHM of the peak in the reflection spectrum. The limit of detection would increase about 8 % with divergence angle of 1.5 *mrad*.

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