Analysis of the Fluorescence Imaging of Surface Plasmon-coupled Emission Microscopy

X. Ji[1], **T. Suyama**[2], **A. Matsushima**[1], **Y. Zhang**[3], **and Y. Okuno**[4]

[1]Graduate School of Science and Technology, Kumamoto University, Kumamoto, Japan
[2]Akashi National College of Technology, Akashi, Japan
[3]Department of Physics and Electronic Information, Wenzhou University, Wenzhou, China
[4]South China Normal University, Guangzhou, China

Abstract— Surface plasmon-coupled emission microscopy (SPCEM) that makes use of a thin layer of metal deposited on glass slides to efficiently excite fluorophore and to collect the emission light is a widely used imaging technique that has found a number of applications in areas such as biotechnology and biological measurements. SPCEM is a relatively new imaging technique that potentially promises better detection sensitivity and higher signal-to-noise ratio than conventional total internal reflection fluorescence (TIRF) imaging. However, recent theoretical studies suggest that the sensitivity of SPCEM is actually reduced due to the metal layer. We have designed a metal-dielectric multilayer film structure which consists of Ag-Si_3N_4 layers, and we have analyzed the transmission of two counter-propagation lights from the multilayer film by using the characteristic matrix method in the film optics. In this paper, optimization is utilized in the design of the multilayer film structure. And it is coated on the glass slides to improve the quality of microscopic imaging. We mainly use the optimization method to design the thickness of the metal layer and the dielectric layer. We analyze the transmission coefficient of two counter-propagation lights from the multilayer film with different thickness and different number of layer. The refractive indexes with different number of the layer of multilayer film are also discussed.

1. INTRODUCTION

Metallic devices at the nanometer scale have stimulated a broad range of interests in nanophotonics and near-field microscopy. For instance, sharp aperture less metal tips have been used to enhance the signal-to-noise ratio and the spatial resolution in a wide range of experiments. Surface plasmon-coupled emission microscopy (SPCEM) was proposed as a high sensitivity technique that made use of a thin layer of metal deposited on glass slide to efficiently excite the fluorescence molecules and to collect the emission light. The SPCEM technique with the total internal reflection fluorescence microscopy (TIRFM) configuration is a widely used imaging technique that has found a number of applications in areas such as biotechnology and biological measurements. The SPCEM method was first proposed as a high sensitivity and efficient fluorescence detection method by Lakowicz et al. [1, 2]. They showed that, through imaging fluorophores near to a silver-coated glass substrate, that the fluorescence emission was highly directional and this increased the collection efficiency to nearly 50%, as well as contributed to low background noise. Experiments have also shown that the fluorescence of single molecules in the vicinity of a thin metallic film may appear enhanced up to a factor of 12 when detected through the metallic film [3, 4].

SPCEM is a relatively new imaging technique that potentially promises better detection sensitivity and higher signal-to-noise ratio than conventional total internal reflection fluorescence (TIRF) imaging. However, recent theoretical studies suggest that the sensitivity of SPCEM is actually reduced due to the metal layer. Duo to the SPCE, the single-layer metal film coated on the surface of glass slide in the TIRFM could intensify the transmission of the excitation light so that the emission of fluorescence molecules was enhanced. On the other hand, the metal film also collected the emission light which propagating direction was contrary to the propagating direction of the excitation light, so that the collection ability to the emission light was lower than that without the metal film. To overcome this disadvantage and further increase the intensity of fluorescent image, we have proposed a metal-dielectric multilayer film structure which consists of Ag-Si_3N_4 layers, and analyzed the transmission of two counter-propagation lights from the multilayer film by using the characteristic matrix method in the film optics. The transmissions of two counter-propagating lights can be enhanced by use of the proposed multilayer film and consequently the fluorescent imaging intensity is larger than that by use of a single-layer metal film in the TIRFM [5, 6]. In this paper, optimization is utilized in the design of the multilayer film structure. And it is coated on the glass slides to improve the quality of microscopic imaging. We mainly use the optimization method to design the thickness of the metal layer and the dielectric layer. We analyze the transmission coefficient of two counter-propagation lights from the multilayer film with different thickness and different number of layer. The refractive indexes with different number of the layer of multilayer film are also discussed.

2. MODEL AND FORMULATE

For simplicity, we calculated the transmission coefficients of plane wave passing through the multilayer film using the transfer matrix method. For concreteness, we assumed that the composite film consists of a Si_3N_4 dielectric layer with the dielectric constant $\varepsilon_d = 4.08$ and an Ag metal-layer (as shown in Fig. 1) and the thickness of metal and dielectric layers were denoted as t and h respectively. Both metal and dielectric are assumed nonmagnetic (i.e., $\mu = 1$). The glass slide in the TIRFM was on top of the composite film and water is below the composite film, fluorescence molecule was in water and in the vicinity of the interface between the composite film and water. We assumed excitation light with 532 nm wavelength propagates along the route of glass→composite film→water, while emission light with 560 nm wavelength propagates along the route contrary to the excitation light. (n_g, n_w, n_m, n_d) denote the refractive indices of glass slide, water, metal,and dielectric, respectively.

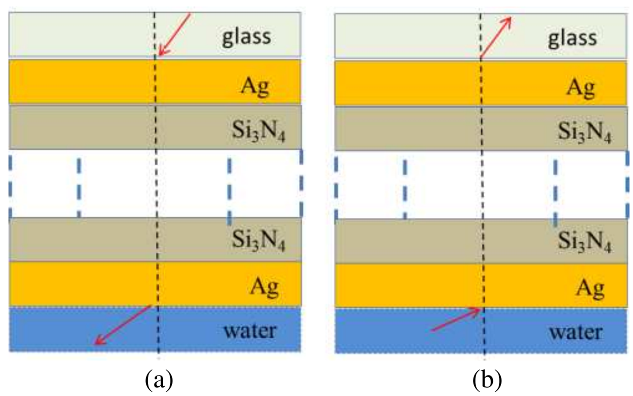

Figure 1: Metal-dielectric photonic crystal structure. (a) The incidence of excitation flight from the glass and (b) the incidence of emission light from the glass.

According to the film optics, the electric-filed transmission coefficient of the film can be written as

$$t_s = \frac{2p_g}{\left(m'_{11} + m'_{12}p_w\right)p_g + \left(m'_{21} + m'_{22}p_w\right)}, \tag{1}$$

where

$$p_g = n_g \cos\theta_g,\ p_w = n_w \cos\theta_w,\ p = n\cos\theta \tag{2}$$

$$M(z) = \begin{bmatrix} m'_{11} & m'_{12} \\ m'_{21} & m'_{22} \end{bmatrix} = \begin{bmatrix} \cos(k_0 nz\cos\theta) & -\frac{i}{p}\sin(k_0 nz\cos\theta) \\ -ip\sin(k_0 nz\cos\theta) & \cos(k_0 nz\cos\theta) \end{bmatrix} \tag{3}$$

for the TE-polarized wave incident on the composite film and

$$t_p = \frac{n_s}{n_l}\frac{2q_g}{\left(m'_{11} + m'_{12}q_w\right)q_g + \left(m'_{21} + m'_{22}q_w\right)} \tag{4}$$

where

$$q = \cos\theta/n,\ q_g = \cos\theta_g/n_g,\ q_w = \cos\theta_w/n_w \tag{5}$$

$$M(z) = \begin{bmatrix} m'_{11} & m'_{12} \\ m'_{21} & m'_{22} \end{bmatrix} = \begin{bmatrix} \cos(k_0 nz\cos\theta) & -\frac{i}{q}\sin(k_0 nz\cos\theta) \\ -iq\sin(k_0 nz\cos\theta) & \cos(k_0 nz\cos\theta) \end{bmatrix} \tag{6}$$

for the TM-polarized wave incident on the MDPC film. The superscripts s and p denote the components for the TE- and TM-polarized lights, respectively.

3. NUMERICAL RESULTS

It is well known that when the TE-polarized light is incident on a metal film, there is no the SPCE to come into being. Only for the TM-polarized light is incident on a metal film, the SPCE may be generated. Therefore, the TM-polarized light or radially polarized light are usually used in the TIRFM to effectively produce the SPCE. In the following calculations, we study only the TM-polarized light.

3.1. Single Layer Metal Film

As a starting point, we first calculate the transmission coefficients from a single-layer Ag film. Figure 2 presents the maximum amplitudes of transmission coefficients as a function versus the thickness of the metal film.

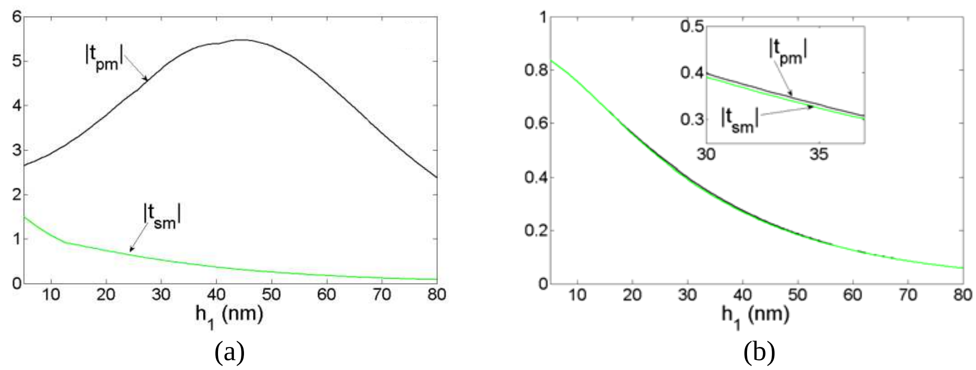

Figure 2: Maximum amplitudes of transmission coefficients as a function versus the thickness h_1 of the metal film. (a) For the excitation light propagating along the glass→metal→water direction; (b) for the emission light propagating along the water→metal→glass direction.

In the Figure 2(a), we can see that, when TM-polarized light is incident on the film, the size of peak value has a non-monotone behavior with the thickness of the film, and reaches its maximum value. When TE-polarized light is incident on the film, the peak value decreases with the thickness. In the Figure 2(b), the peak value decreases with the thickness and all are smaller for larger thickness.

3.2. Metal-dielectric-metal Composite Film

In Figure 3, we can see that, when the thickness of the metal film within a certain range, the transmission coefficients of two counter-propagating lights all are larger than 1, in another word, the transmissions of two counter-propagating lights are both enhanced.

Figure 4 presents the amplitudes of transmission coefficients and phases of the composite film as a function versus the incident angles. As is shown in the Figure 4, transmission coefficients of two counter-propagating lights all are larger when the incident angle is less than 74 degrees.

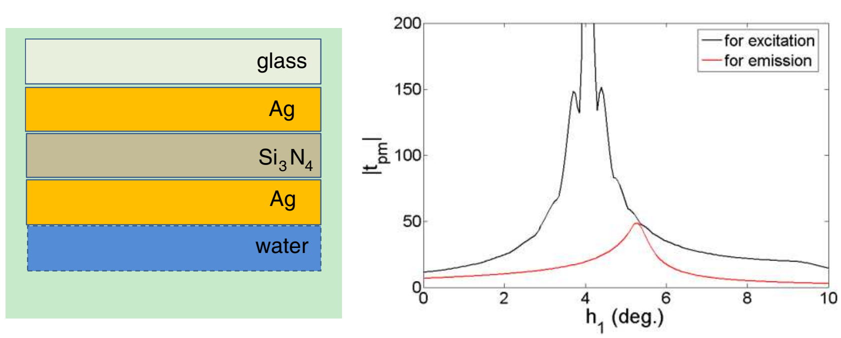

Figure 3: Maximum amplitudes of transmission coefficients of the metal-dielectric-metal sandwich film as a function versus the thickness h_1 of the metal film when $h_2 = 37\,\text{nm}$. Black and red curves are the cases for the excitation light propagating along the glass→sandwich→water direction and for the emission light propagating along the water→sandwich→glass direction, respectively.

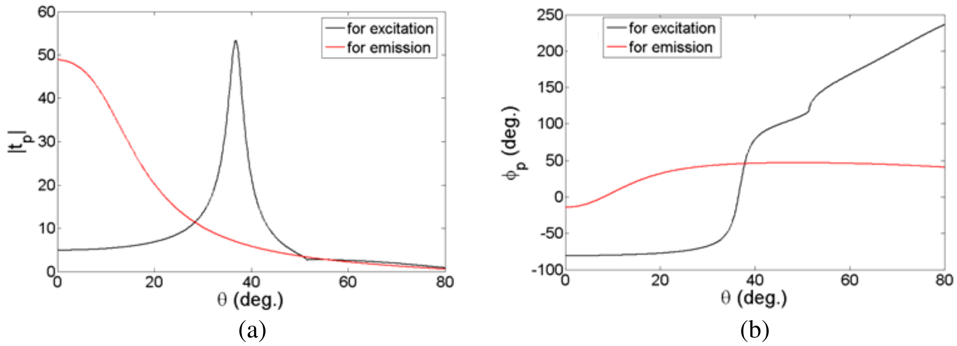

Figure 4: (a) Amplitude and (b) phase of transmission coefficients of light passing the sandwich versus the incident angle when $h_1 = 5.25\,\text{nm}$ and $h_2 = 37\,\text{nm}$.

3.3. Multilayer Metal-dielectric Composite Film

Figure 5 presents the amplitudes of transmission coefficients of the composite film as a function versus the thickness of the metal film when the thickness of the dielectric film is 28 nm. When the thickness of the metal film is within a certain range, the bidirectional transmission coefficients are greater than 1. As shown as Figure 6, as $\theta < 70^\circ$, both the transmission coefficient of emission light and excitation light are very large. Compared to metal-dielectric-metal sandwich film, the maximum amplitudes of transmission coefficients decreased. However, as $\theta < 46.5^\circ$, transmission lights of both directions were strengthened at the same time. And in this incident Angle range, phase change is very small.

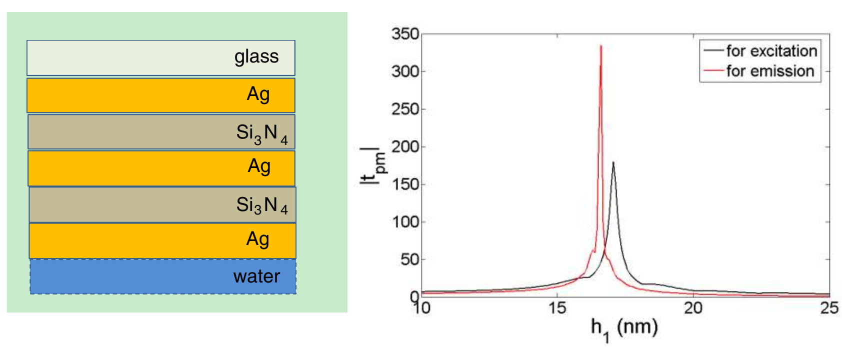

Figure 5: Maximum amplitudes of transmission coefficients of the composite film as a function versus the thickness h_1 of the metal film when $h_2 = 28\,\text{nm}$. Black and red curves are the cases for the excitation light propagating along the glass→composite film→water direction and for the emission light propagating along the water→composite film→glass direction, respectively.

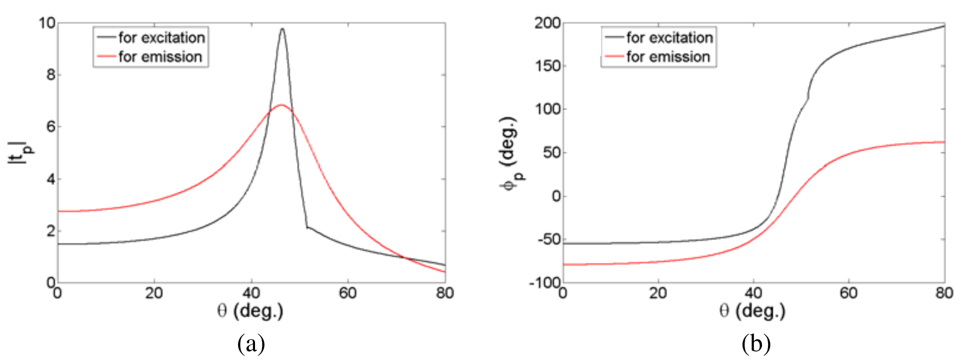

Figure 6: (a) Amplitude and (b) phase of transmission coefficients of light passing composite film versus the incident angle when $h_1 = 13\,\text{nm}$ and $h_2 = 27\,\text{nm}$.

4. CONCLUSION

To enhance the image of single molecule fluorescence, one single-layer metal film is usually deposited on the glass slide in conventional localized TIRFMs. In this paper, we propose two kinds of metal-dielectric multilayer film, and it is coated on the glass slides to improve the quality of microscopic imaging. We analyze the transmission coefficient of two counter-propagation lights from the multilayer film with different thickness and different number of layer. The refractive indexes with different number of the layer of multilayer film are also discussed. It is clear that the transmissions of multilayer metal-dielectric composite film for two counter-propagating lights are both enhanced. The brightness of fluorescent imaging patterns will be increased remarkably if such multilayer metal-dielectric composite film is used in the total internal reflection fluorescence microscopy.

REFERENCES

1. Török, P., P. Varga, Z. Laczik, and G. R. Booker, "Electromagnetic diffraction of light focused through a planar interface between materials of mismatched refractive indices: An integral representation," *J. Opt. Soc. Am. A*, Vol. 12, 325–332, 1995.
2. Lakowicz, J. R., "Directional surface plasmon-coupled emission: A new method for high sensitivity detection," *Biochem. and Biophys. Res. Comm.*, Vol. 307, No. 3, 435–439, 2003.
3. Van de Nes, A., L. Billy, S. Pereira, and J. Braat, "Calculation of the vectorial field distribution in a stratified focal region of a high numerical aperture imaging system," *Opt. Express*, Vol. 12, 1281–1293, 2004.
4. Tang, W. T., E. Chung, Y. H. Kim, P. T. C. So, and C. J. R. Sheppard, "Investigation of the point spread function of surface plasmon-coupled emission microscopy," *Opt. Express*, Vol. 15, No. 8, 4634–4646, 2007.
5. Suyama, T., X. Ji, and Y. Zhang, "A metal-dielectric composited film applied to enhance the fluorescence imaging," *PIERS Proceedings*, 1409–1414, Stockholm, Aug. 12–15, 2013.
6. Suyama, T., X. Ji, and Y. Zhang, "Enhancement of fluorescent labeling via a composited thin film," *International Journal of Polymer Science*, No. 921489, 2014.