Published in final edited form as:

Thin Solid Films. 2006 July ; 510(1-2): 15–20. doi:10.1016/j.tsf.2005.07.312.

Waveguide-modulated surface plasmon-coupled emission of Nile blue in poly(vinyl alcohol) thin films

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Abstract

Surface plasmon-coupled emission (SPCE) phenomenon is the coupling of excited fluorophores near a silver film with surface plasmons, resulting in directional emission into the underlying glass substrates. We report a complex coupling of Nile Blue fluorophore with 50 nm silver mirror, resulting in emission at several angles in the glass substrate, with either s or p polarization. This complex pattern of directional and polarized emission appears to be due to optical waveguide effects occurring when the sample thickness becomes comparable to the emission wavelength. We expect waveguide-modulated SPCE to have applications to biophysics and sensing.

Keywords

Surface plasmon-coupled emission; Waveguide; Silver film; Nile Blue

1. Introduction

Fluorescence detection is widely used in biological research and increasingly in medical diagnostics and biotechnology. Most of these applications rely on the free-space emissive properties of fluorophores. By free-space we mean emission into essentially isotropic transparent media. For the past several years we have been working on modification of the emissive properties of fluorophores due to near-field interactions with metallic surfaces or particles [1,2]. We refer to this metal-enhanced fluorescence as radiative decay engineering (RDE) because the far-field effects are “engineered” to best serve the particular applications. The first developments of RDE were to obtain increased fluorescence intensities and increased photostability due to proximity of fluorophores to sub-wavelength sized silver particles [3–5].

More recently we began studies of fluorophores near thin silver films [6,7] comparable to those used for surface plasmon resonance (SPR). SPR is the absorption of light by a thin metal film, typically silver or gold, when the wavevector of the p-polarized incident light matches the wavevector of the surface plasmons at sample-metal interface [8–11]. Matching of the wavevector requires the light incident on the metal pass through a prism with high

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refractive index. The angle of incidence through the prism must be adjusted to match the wavevector of the incident light and of the surface plasmons in metal-sample interface.

Surface plasmon-coupled emission (SPCE) phenomenon is related to SPR, however the emission is detected rather than absorption. Excited dipoles of fluorophores near the metal surface couple to the surface plasmons. The emission through the coupling glass appears to be due to radiating surface plasmons, but the emission spectra of the plasmons match the free-space emission spectra of the fluorophores, at least at our current level of resolution. The coupled emission occurs at a sharply defined angle from the normal, and this angle is equal to the surface plasmon angle for the emission wavelength. Although the direction of SPCE can be roughly predicted from the SPR equations, the theory of SPCE has been recently presented [12]. This elegant theory explains the SPCE observed for thin samples and predicts more than one SPCE peak that couple out, due to the possible presence of more than one resonance mode. One of the goals of this manuscript is to provide quantitative data which can be used by theoreticians to verify their predictions.

It is important to notify that fluorophore-metal coupling is a near-field phenomenon occurring without an emission of the photon, similar to the Forster resonance energy transfer. The amount of the far-field emission transmitted at the SPCE angle is minimal comparing to SPCE.

The SPR as well as SPCE angles depend on the thickness of the sample adjunct to the metal surface. This effect is used in SPR-based instruments to study the macromolecular binding to surfaces and in SPCE studies of bioassays [13–16]. Recently, Salamon and others proposed a new spectroscopic tool based on coupled plasmon-waveguide resonators [17] and demonstrated its usefulness in SPR measurements of ligand binding to the human β 2-adrenergic receptor [18]. Multiple surface plasmons emitting from samples with various thickness have been also observed [19,20]. The measurements presented in this manuscript are complementary to those reported in Ref. [19]. We believe, that an extension to near infrared region will make the SPCE technique more attractive. In waveguide resonators both modes of polarization, p and s, can propagate. Therefore, the SPR waveguide-based experiment can use p- or s-polarized light beams. In SPCE waveguide-based experiment the directional emission can also be p- or s-polarized. These radiations appear at different angles.

In this manuscript we demonstrate the Nile Blue SPCE in waveguide-like conditions.

2. Materials and methods

2.1. Sample preparation

Microscope glass slides were coated by vapor deposition by EMF Corp. (Ithaca, NY). A 50 nm thick layer of silver was deposited on the glass with about 2 nm chromium undercoat. Fluorophores were deposited on the surface by spin coating at 3000 rpm a solution of low-molecular weight poly(vinyl alcohol) (PVA, MW. 13,000–23,000; Aldrich) in water. The PVA solutions contained 5×10^{-4} M Nile Blue from Exciton. By using 2% and 10% PVA

solutions we targeted the thickness of sample films 50 nm [21] and above 500 nm, later called the thin and thick samples, respectively.

2.2. Fluorescence measurements

The spin coated slides were attached to a hemi-cylindrical prism made of BK7 glass. This combined sample was positioned on a precise rotary stage which allows excitation and observation at any desired angle relative to the vertical axis around the cylinder. For excitation we used the reversed Kretschmann (RK) configuration (Scheme 1). In this configuration the sample was excited from the air or sample side which has a refractive index lower than the prism. In this case it is not possible to excite surface plasmons. The angle of incidence does not matter but we used normal incidence.

There is another possible configuration for the excitation called Kretschmann or attenuated total internal reflection. In this configuration the p-polarized excitation beam goes through the glass prism at the SPR angle and induces surface plasmons at the metal-dielectric interface. The evanescent wave of these plasmons penetrates the dielectric layers adjunct to the metal and excites fluorophores, which are present there.

We have already shown that the angular distribution of the SPCE intensity does not depend on the chosen configuration [7,19]. However, the photographs of SPCE cones look better when taken with RK configuration.

Observation of the angular distribution of the emission was performed with a 3 mm diameter fiber bundle, covered with a 200 μm vertical slit, positioned about 15 cm from the sample. This corresponds to an acceptance angle below 0.1° . For the spectra measurements the 200 μm slit was removed from the fiber and fiber was placed at close proximity to the sample. The output of fiber was directed to 8000 SLM spectrofluorometer (SLM, Inc.).

For excitation we used the 615 nm output of cavity-dumped DCM (4-dicyanmethylene-2-methyl-6-(*p*-dimethyl-aminostyryl)-4*H*-pyran) dye laser pumped by a modelocked argon ion laser, 76 MHz-repetition rate, 120 ps half-width. Scattered light at 615 nm was suppressed by observation through a cut-off 630 nm glass filter. For the photographs the samples were attached to a hemi-spherical prism rather than a hemi-cylinder.

2.3. Reflectance calculations

The reflectance profiles of the PVA-Ag-BK7 substrates were calculated with TFCalc. 3.5 software (Software Spectra, Inc., Portland, OR). The thicknesses of the samples were estimated by comparing the measured SPCE with the calculated reflectance for the emission wavelength.

2.4. Theory

Based on our previous studies [6,7] we believe that SPCE is essentially the same phenomenon of SPR, and will be found for those conditions where the reflectivity of the metal is at a minimum. The theory for the reflectivity of multi-layer dielectric and metal films is complex and the subject of monographs focused on surface plasmons [22,23] or the closely related topics of thin film optical filters [24] or optical waveguides [25,26]. It would

be difficult to describe these complex optics in a few brief paragraphs. Instead, we describe SPR/SPCE using the example of a single reflectivity minimum without waveguide effects. The extension to multiple angle SPCE is then intuitively understandable.

SPR occurs when light is incident on a thin metal film through a medium of moderate refractive index such as glass. Light absorption by the silver film occurs when the angle of incidence (θ_I) equals the SPR angle for the incident wavelength (θ_{SP}). SPR does not occur if the light is incident on the metal from the side with lower refractive index, in our case a PVA film. However, SPCE does occur without the creation of surface plasmons. Absorption by surface plasmons at a silver-sample interface occurs when the wavevector of the light incident through the prism matches the wavevector of the surface plasmon, both for the in-plane components. The free-space wavevector of the incident light in prism is given by

$$k_I = \frac{2\pi}{\lambda} = \frac{n_p \omega}{c} = n_p k_0 \quad (1)$$

where n_p is the refractive index of the prism, $\lambda = \lambda_0 / n_p$ is the wavelength in the prism, λ_0 is the wavelength in a vacuum, ω is the frequency in radians/sec, and $k_0 = 2\pi / \lambda_0$ is the wavevector or propagation constant in a vacuum. The wavevector of the incident light in plane of the silver-sample interface is given by

$$k_x = k_0 n_p \sin \theta_I. \quad (2)$$

Calculation of the wavevector for the surface plasmon is more complex. The dielectric constant (ϵ) of a metal (m) is a complex quantity given by

$$\epsilon_m = \epsilon_r + i\epsilon_m \quad (3)$$

where $i = \sqrt{-1}$ and the subscripts indicate the real (r) and imaginary (im) components. These constants are wavelength (frequency) dependent, but this effect is minor over the limited wavelength range of our experiments. For silver we used $\epsilon_m = -19 + 0.6i$ at 665 nm (TFCalc. 3.5 software, Software Spectra, Inc., Portland, OR). The wavevector for the surface plasmon is given by

$$k_{SP} = \frac{\omega}{c} \left(\frac{\epsilon_m \epsilon_s}{\epsilon_m + \epsilon_s} \right)^{1/2} = \frac{\omega}{c} \left(\frac{\epsilon_r \epsilon_s}{\epsilon_r + \epsilon_s} \right)^{1/2} \quad (4)$$

where ϵ_m is the dielectric constant of the metal (m) and ϵ_s is the effective dielectric constant of the multi-layer structure that includes sample (s). The value of k_{SP} is often approximated using the real part of the metal dielectric constant (ϵ_r).

The condition for SPR absorption is satisfied when

$$k_{SP} = k_x = k_0 n_P \sin \theta_{SP}. \quad (5)$$

In reality SPR does not occur at a single angle, but over a relatively narrow range of angles determined by the optical constants and resonance response of the metal. Also, Eq. (5) is appropriate only for p-polarized incident light. By analogy, we expect SPCE to occur at the plasmon angle for the emission wavelength.

Eqs. (1)–(5) describe the surface plasmon angles for either thin or infinitely thick samples, for p-polarized light. For calculations of the entire angle-dependent reflectivity curves for thin samples, or samples with thicknesses comparable to the emission wavelength, we used a software package, TF Calc., which is used to design multi-layer optical filters.

3. Results

We first examined the thin PVA sample (2%) with a targeted thickness of 50 nm. Fig. 1, top shows the emission intensity of Nile Blue observed for all accessible angles. The emission intensity through the prism peaked sharply at 49°. The emission intensity was not significant at other angles on the prism side of the samples. Using Eqs. (1)–(5) with $n_s = 1.50$ for PVA results in a calculated value of $\theta_{SP} = 49^\circ$ for a 44 nm thick sample at 665 nm. This supports our earlier observation that the angles of SPCE coincide with the angles of minimum reflectivity for the metal surface [6,7].

In addition to the SPCE it is possible to observe free-space emission from Nile Blue on the side distal from the prism. This emission is weak (Fig. 1, top), but increases in intensity for the thicker PVA sample (Fig. 1, bottom). This result is consistent with weaker coupling of Nile Blue with the metal surface when more distant from the metal surface. The presence of free-space emission provides the opportunity to compare the polarizations of both emissions. For the thin sample the s- and p-polarized free-space emission shows an intensity ratio near 2 (Fig. 2, top), which is typical of fluorophores in viscous or solid media. In contrast, the SPCE of Nile Blue is almost completely p-polarized (Fig. 2, bottom). The ratio of the p to s intensities is near 30. Such highly polarized emission is not possible for photoluminescence of an isotropic sample [27]. This high polarization is consistent with emission of surface plasmons into the prism since wavevector matching is only possible for p-polarized light.

It is interesting to explore the symmetry of SPCE. Consider an excited fluorophore above a metal surface. The fluorophore can “feel” the same surface plasmons at all azimuthal angles around the normal axis. Hence we expected SPCE to occur not just at a single angle, but as a cone around the normal (Scheme 1, bottom). We used a glass hemisphere as the prism to examine the cone-of-emission, which was projected onto a white tracing paper and photographed with a digital camera (Fig. 3). The SPCE is p-polarized at all angles around the cone irrespectively on the polarization of excitation. The appearance of the cone does not require the incident light to have normal incidence, but is seen for any angle of incidence.

We next investigated the SPCE with the thicker sample (Fig. 1, bottom). Instead of SPCE at a single angle, SPCE was observed at 4 angles (Table 1). Additionally, the rings displayed

alternative s and p polarizations. This can be seen from the photographed cone-of-emission for the thick sample (Fig. 4, top). Only the two most intense rings are visible in the photograph. The outer ring is p-polarized and the inner ring s-polarized, as can be seen from photographs taken through a polarizer (Fig. 4, bottom).

We considered the possibility that the multiple SPCE rings with different polarizations were related to waveguide modes in the thicker PVA sample. Fig. 5 shows the calculated reflectivity curves. For these calculations we varied the assumed thickness of PVA to match the reflectivity minima with the observed SPCE angles. For an assumed thickness of 44 nm, the thin sample, we calculated a single minimum at 49° for p-polarized light. For an assumed thickness of 595 nm we calculated 4 reflectivity minima, with alternating s and p polarizations (Fig. 5). The angles and polarizations precisely matched those observed (Table 1). We conclude that the waveguide modes present in the thick PVA samples are the origin of the multiple rings and polarizations of the coupled emission.

4. Discussion

SPCE in optical waveguides can have numerous practical applications. The SPCE angles, polarizations and intensities are sensitive to the sample thickness. Hence any process which alters the sample thickness or affects refractive index will change the angles. The effective sample thickness could be changed by vapor binding to a polymer, by biomolecule binding to a bioaffinity surface, or by changes in ionic strength, to name a few. Such changes could be measured with point or imaging detectors. Alternatively, the waveguide-modulated SPCE could be used to measure the polymer thickness.

Another advantage of SPCE with optical waveguides is the wealth of information available for the optical properties of thin layered films [23–25]. The close connection between reflectivity and SPCE will facilitate the development of SPCE devices by predicting the optical properties of the films.

Also, from practical point of view, the waveguide-modulated SPCE could be more convenient because it offers the narrow peaks at smaller angles. Such radiation can be more precisely detected without using a high refractive index glass prism.

Acknowledgments

This work was supported by the National Center for Research Resources, RR-08119, and the Biomolecular Interaction Technologies Center (University of New Hampshire).

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Fig. 1. Angular distribution of RK/SPCE for the thin (2% PVA, top) and thick (10% PVA, bottom) Nile Blue samples. The 615 nm excitation was p-polarized. The emission at 665 nm was observed through the fiber mounted on a precise rotary stage. The polarization and intensities of the modes are summarized in Table 1.

Fig. 2.

Emission spectra of Nile Blue for thin PVA film, with p-polarized excitation and emission observed through p or s oriented polarizer. Top, free-space emission measured at 150° . Bottom, SPCE measured at 49° (see Fig. 1).

Fig. 3. Color photograph of SPCE from Nile Blue projected onto white tracing paper. The photos were taken through a 630 nm long wave pass emission filter. Top, front view. Bottom, side view.

Fig. 4. Color photograph of SPCE for the thick sample. Top, no emission polarizer. Bottom, s or p emission polarizer. The two most intense rings are seen at 51.5° and 64° .

Fig. 5.

Reflectivity calculations for 665 nm for a 44 nm thick PVA sample (top) and a 595 nm thick PVA sample (bottom). The calculations were performed for four-phase system: glass ($n_p=1.514$), 50 nm silver ($\mathbf{e}_m=-19+0.6i$), 44 or 595 nm PVA ($n_s=1.50$), and air ($\mathbf{e}_0=1.0$).

Scheme 1.

Top: Reverse Kretschmann configuration, RK. The excitation directly reaches the sample and does not excite surface plasmons. The fluorophores can emit into free-space (---, RK/FS) or couple through surface plasmon to the glass prism (RK/SPCE). Bottom: Cone-of-emission expected for SPCE.

Table 1

Experimental (Exp) and calculated (Calc) reflectivity properties for the thin and thick PVA samples on a 50 nm silver film

Thickness		Angle (deg)		Polarization		Intensity	
Exp	Calc	Exp	Calc	Exp	Calc	Exp	Calc
Thin	44 nm	49°	49°	p	p	1	1
Thick	595 nm	42°	42.5°	p	p	0.25	0.6
		51.5°	52°	s	s	0.68	0.9
		64°	63.5°	p	p	1.0	1.0
		72°	71°	s	s	0.07	0.4

The calculations were performed for four-phase system: glass ($n_p = 1.514$), 50 nm silver ($\epsilon_m = -19 + 0.6i$), 44 or 595 nm PVA ($n_s = 1.50$), and air ($\epsilon_0 = 1.0$).