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Surface plasmon-coupled directional fluorescence emission

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Abstract

Directional fluorescence emission of a sulforhodamine 101 in polyvinyl alcohol film has been observed from samples deposited on semi-transparent silver mirror. The fully p-polarized fluorescence emerges through the glass prism in form of hollow cone. The angle of this cone of emission depends on the thickness of the sample, and does not depend on the mode of excitation. The angular dependence of surface plasmon-coupled emission (SPCE) on the sample thickness has been discussed as well as its relevance to the surface plasmon resonance (SPR) analysis.

Keywords

surface plasmon-coupled emission; silver film; directional emission

1. INTRODUCTION

Surface plasmon resonance (SPR) is widely used to measure bioaffinity reactions. 1–3 SPR is based on the absorption of light by thin (about 50 nm) gold films on a glass prism. Light incident on the film through the prism displays strong absorption at a specific angle of incidence, known as the surface plasmon angle (θ_{SP}). When light absorption occurs there exists an evanescent field in the air or water phase above the gold, which extends about 200 nm into this distal phase. The surface plasmon angle θ_{SP} is dependent on the effective refractive index of this phase. Binding of macromolecules within the evanescent field results in small changes in the local refractive index, which can be detected by change in θ_{SP} . The change in refractive index is due to the mass of the biomolecules and their effect on the refractive index. As a result SPR analysis is usually performed to detect bioaffinity reactions using unlabeled biomolecules.

The occurrence of SPR requires matching of the wavevectors of the incident light and of the surface plasmons at the gold-sample interface. Plasmons cannot be excited by illumination through water due to an inability to match the wavevectors at the gold-sample interface. Matching of the wavevectors requires illumination of the metal through a medium with a higher refractive index, typical glass.^{4–7} SPR only occurs for the p-polarized component of the incident light,^{8–12} for which it is possible to match the wavevector by changing the

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incidence angle. The term “p” refers to the electric vector of the incident light oriented in the plane of incidence.

In several recent reports we described an emissive process that seems to be related to SPR.^{13–16} We placed fluorophores in close proximity to 50 nm thick silver or gold films on glass prisms. Such metal films are optically dense and appear to be nearly opaque. The fluorophores were then excited either through the air/water phase or through the glass prism and metal film. In both cases we observed a bright emission, which passed through the silver or gold film and back into the prism. Remarkably, this emission in the prism occurred only at a specific angle relative to the normal axis, that is, the emission was directional. Since the sample was symmetrical around the normal axis, the emission in the prism appeared as a cone of emission around this axis. The emission angle was consistent with the surface plasmon angle calculated for the emission wavelength. Additionally, the emission seen through the prism was completely p-polarized. We refer to this phenomenon as surface plasmon-coupled emission (SPCE). We believe the directional energy is due to a near field interaction of the excited fluorophore with the metal film, which excites surface plasmons. These plasmons then emit to yield the far field radiation.

As we continue our studies of SPCE we decided to examine the dependence of the emission angle (θ_F) on the thickness of the sample. Such dependence should be complementary to the SPR measurements.

2. MATERIALS AND METHODS

2.1 Sample preparation

Quartz slides (Spectrosil I, optical flatness $\lambda/4$; Starna Cells Inc.) were coated by vapor deposition by EMF Corp. (Ithaca, NY). A 50 nm thick layer of silver was deposited on the quartz with about 2 nm chromium undercoat. Fluorophores were deposited on the surface by spin coating at 3000 rpm a solution of low-molecular weight polyvinyl alcohol (PVA, MW. 13,000–23,000; Aldrich) in water. The PVA solution contained sulforhodamine 101 (S101) from Aldrich. In order to achieve various thicknesses of the sample layer silvered and unsilvered slides were spin coated with 0.5, 1.0 and 2.0 % PVA solutions containing S101. The ratio S101/PVA was kept constant.

Initial estimates of the PVA film thickness were made by measurements of the optical densities of S101 on the spin coated slides. The optical densities were low and not reliable when measured on the silver coated slides. Hence we measured the optical densities of samples spin-coated on unsilvered quartz slides. Control experiments measuring the optical density of S101 washed off the slides showed equal amounts of S101 remaining on the quartz or silvered surfaces. By comparison of the optical densities of the spin coated slides with optical densities of films prepared by evaporation of the same PVA solutions we estimated PVA thicknesses to be 15, 30 and 53 nm for 0.5, 1.0 and 2.0 % PVA, respectively. Due to very small optical densities these values are only rough approximations. We believe the actual thicknesses are those found from comparison of the angular SPCE distributions and calculated reflectances simulated for the experimental conditions.

2.2 Fluorescence measurements

The spin coated quartz slides (0.7 mm thick, $n=1.46$) were attached to a hemi-cylindrical prism made of BK7 glass, $n = 1.52$ (Fig. 1). The refractive indexes were matched with glycerol, $n = 1.475$. 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 [15]. Two modes of excitation are possible (Fig. 1). The sample could be excited through the prism (Fig. 1, top). In this case the incident light was completely reflected at all angles except when the incident angle θ_i equaled the surface plasmon angle θ_{sp} . For incident angles near θ_{sp} there exists an evanescent wave in the air-sample side, distal from the incident light. This evanescent field extends about 200 nm into the air or sample. This field is enhanced about 20-fold by the resonance interaction. 17–20 This mode of illumination is called the Kretschmann (KR) configuration. The sample can also be 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 and the angle of incidence does not matter, but we used normal incidence (Fig. 1, bottom). This is called the reverse Kretschmann (RK) configuration. In this manuscript we will describe our observations in RK configuration. Observation 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 excitation we used the 514 nm output of a mode-locked argon ion laser, 76 MHz repetition rate, 120 ps half-width. Scattered light at 514 nm was suppressed by observation through a holographic supernotch-plus filter (Kaiser Optical System, Inc., Ann Arbor, MI). Emission intensities were observed through a long wave pass filter LWP 550 nm in addition to the notch filter.

2.3 Theory

We now describe the theory for SPR of thin films coating the metal surface. For such films there is a single resonance at a single angle of incidence and only the p-component of the incident light is absorbed. The observation of SPCE at multiple angles is best understood in terms of this model, which provides a basis for comparison with the thick film results. In the thicker PVA film we found results consistent with optical waveguide effects. The theory for SPR in such waveguides is considerably more complex.

SPR occurs when light is incident on a thin metal film through a medium of moderate refractive index such as glass (Fig. 1, top). Light absorption by the silver film occurs when the angle of incidence (θ_i) equals the SPR angle for the incident wavelength (θ_{sp}). The reflectivity of the metal film is high except for a small range of angles around θ_{sp} . SPR does not occur if the light is incident on the metal from the side with lower refractive index, typically an aqueous sample or in our case a polyvinyl alcohol (PVA) film.

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. The wavevector matching has to occur for the in-plane component of the incident wavevector. This requirement is a result of solving Maxwell's equation for the boundary condition that

the in-plane component of the electric field must be continuous across the interface. The free-space wavevector of the incident light 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, k_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

$$\varepsilon_m = \varepsilon_r + i\varepsilon_{im}, \quad (3)$$

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 $\varepsilon = -14.06 + 0.45i$ at 600 nm and $-9.0 + 0.3i$ at 514 nm, which represent consensus values obtained from several sources.^{14,21–23} To a first approximation the real part of ε_m is determined by the conductivity of the metal and the imaginary part of ε_m is due to attenuation (absorption) of the incident light. The wavevector for the surface plasmon is given by

$$k_{SP} = \frac{\omega}{c} \left(\frac{\varepsilon_m \varepsilon_s}{\varepsilon_m + \varepsilon_s} \right)^{1/2} = k_0 \left(\frac{\varepsilon_m \varepsilon_s}{\varepsilon_m + \varepsilon_s} \right)^{1/2}, \quad (4)$$

where ε_m is the dielectric constant of the metal (m) and ε_s is the effective dielectric constant at the silver-sample (s) interface. Because the real part of ε_m is larger than the imaginary part the wavevector can be approximated by

$$k_{SP} = k_0 \left(\frac{\varepsilon_r \varepsilon_s}{\varepsilon_r + \varepsilon_s} \right)^{1/2}. \quad (5)$$

Using the optical constants for silver $\varepsilon_m = -9.0 + 0.3i$ at 514 nm,^{21–24} $\varepsilon_s = 1.0$ for the air and $n_p = 1.52$ for the BK7 glass prism one can calculate $\theta_{SP} = 44.14^\circ$ from eq. 4 and $\theta_{SP} = 44.25^\circ$ from eq. 5.

The condition for SPR absorption is satisfied when

$$k_{SP} = k_x = k_0 n_p \sin \theta_{SP}. \quad (6)$$

Equation 6 illustrates an important property of surface plasmon resonance. For SPR to occur the electric vector of the incident light in the metal plane needs to be modified by the angle of incidence. This only occurs if the electric vector is p-polarized (Fig. 1). 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. Calculation of the angle-dependent reflectivity curves requires moderately complex equations or web-based software.

25

3. RESULTS AND DISCUSSION

We measured the emission intensities for all accessible angles relative to the normal axis. For PVA thicknesses obtained from 0.5, 1.0 and 2.0 % PVA solution we observed a single angle of maximum S101 intensity (Fig. 2, top). The angle is larger for 2.0 % versus 1.0 and 0.5 % of PVA. This dependence is similar to that found for SPR, where the SPR angle increases when the effective dielectric constant increases. Since the occurrence of angular SPCE does not depend on the mode of excitation, there is no reason for SPCE to occur at any specific azimuthal angles (Fig. 3). We examined the azimuthal distribution of SPCE by projecting the SPCE onto white tracing paper. We observed a cone of emission, which was symmetric around the normal axis, as seen in the photograph (Fig. 4, top). Scattered excitation light was eliminated with a long pass filter. The bottom of Fig. 4 shows the emission spectrum, which appears to be highly p-polarized. We note that the samples were excited in the RK configuration, for which the incident light cannot excite surface plasmons in the metal film. The plasmonic interaction is being induced by excited fluorophores. At present we believe the emission is due to surface plasmons in the metal, which result from near-field coupling with the excited fluorophores. The angle at which SPCE emerges should approximately correspond to the minimum reflectance angle for the emission, not excitation wavelength.

The reflectance profiles for samples obtained from 0.5, 1.0 and 2.0 % PVA are shown in Fig. 2, bottom. These calculations were done for assumed thicknesses adjusted such that the reflectance minima correspond to θ_F . We believe that such estimation provides real values of samples thickness. Left panel of Fig. 5 shows the calculated SPCE angles in function of sample thickness. This dependence was calculated for a five-phase system (Fig. 5, right) corresponding to the described experiment with using commercial software for thin films.²⁶ Similarly to the SPR analysis, the SPCE angle depends on the size of the sample (protein mass) deposited on semitransparent metallic mirror. In the case of SPCE, two configurations are possible, KR and RK (Fig. 1). The KR configuration requires excitation (p-polarized) through the prism and gives possibility of simultaneous measurements of reflectance (SPR) and SPCE at smaller angles ($\theta_F < \theta_I$). Although RK configuration gives weaker (than KR) SPCE signals¹⁵ provides also some benefits. In RK/SPCE configuration there is no need for polarized excitation. Also, the sample can be excited in UV with emission observed in longer wavelengths. Both, SPR and SPCE are very sensitive techniques since angles can be measured very accurately. The combination of SPR and SPCE should further improve this plasmonic-based technique.

Acknowledgments

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Fig. 1. Geometries used for SPCE measurements. Top: for surface plasmon excitation (Kretschmann configuration, KR). The excitation enters through the coupling prism. Bottom: in reverse Kretschmann configuration, RK. The excitation directly reaches the sample and does not excite surface plasmons.

Fig. 2.

Top: angular distribution of the S101 fluorescence emission for 0.5, 1.0 and 2.0 % PVA. Observation was at 600 nm. Bottom: reflectance curves calculated for adjusted thicknesses of the sample to yield appropriate angle of the minimum reflectance (maximum emission).

Fig. 3.
Cone of emission for SPCE in RK configuration.

Fig. 4.

Top: front (left) and side (right) views of cone of emission for S101 in 1 % PVA observed with hemi-spherical prism and RK excitation (see Fig. 3). The emission was incident on tracing paper and photographed through a 550 nm long wave pass filter. Bottom: polarized SPCE spectra of S101 in 1 % PVA spin-coated on 50 nm silver mirror.

Fig. 5.

The dependence of the SPCE angle at 600 nm on the thickness of the sample (left).

Calculations were done for five-phase system corresponding to the experimental conditions (right).