Surface Plasmon-Coupled Ultraviolet Emission of 2,5-Diphenyl-1,3,4-oxadiazole

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We studied surface plasmon-coupled emission (SPCE) of 2,5-diphenyl-1,3,4-oxadiazole (PPD) using a 20 nm aluminum film deposited on a quartz substrate. The directional SPCE UV fluorescence occurs within a narrow angle at 57° from the normal to the coupling hemicylindrical prism. This radiation is almost completely p-polarized, consistent with its origin from surface plasmons. These surface plasmons are induced by excited PPD molecules. The coupling of excited fluorophore dipoles with the aluminum is highly efficient, exceeding 50%. Different fluorescence emission wavelengths are emitted at slightly different angles on the prism, providing intrinsic spectral resolution. SPCE fluorescence on thin aluminum films can be used with many UV absorbing and emitting fluorophores.

Introduction

Surface plasmons are oscillating electrons (electrical charges) on a metallic surface. These resonant oscillations are induced when a thin metal film is illuminated through a glass prism (higher refractive index) and the angle of incidence (θ_1) is appropriate, i.e., projection of the light vector on the interface equals the wavevector of the plasmons. This results in a strong absorption as seen by decreased reflectivity of incident light at the surface plasmon resonance (SPR) angle. SPR analysis (SPRA) is now widely used for detection of bioaffinity reactions on surfaces.^{1–4} This is possible because the SPR angle is sensitive to the refractive index and thickness of the sample above the metal. The penetration depth (evanescent field) of surface plasmons is approximately one-third of the incident light wavelength, which is the appropriate distance for protein, DNA, or membrane studies.

At present, SPRA is performed on slides coated with thin gold films (usually 47–50 nm thick) because of the superior surface performance of gold, higher resistance to the environment, and well-developed surface chemistry. However, SPR measurements with gold films require visible or near-infrared light. Aluminum is known for excellent reflective properties in the UV region.

It is interesting to compare the SPR responses expected for gold and aluminum. Assuming a 15 nm change in sample thickness, we calculated the reflectances for 47 nm gold on a glass substrate and a 650 nm incidence light (Figure 1, left) as well as reflectances for 20 nm aluminum on a quartz substrate and a 300 nm incident beam (Figure 1, right). For both gold and aluminum, the minima of reflectances are well defined. Although the angular dependence of reflectance is sharper for gold, the absolute change in the reflectance angle is 2—3-fold larger for aluminum. We believe that changes in the mass (in the SPR experiments) can be detected easier in the UV using aluminized quartz or sapphire slides.

In our recent reports we described a related phenomenon we called surface plasmon-coupled emission (SPCE).⁵⁻⁸ We ob-

served that excited fluorophores near thin metal films can induce surface plasmons, resulting in directional radiation into the glass substrate. The angular dependence of the radiation and its p-polarization are consistent with radiating surface plasmons, the reverse process of SPR. The spectral properties of SPCE were found to be identical to those of fluorophores, except for complete polarization of the SPCE.

In most of our previous studies we used thin (50 nm) silver^{5–8} or gold⁹ surfaces. Very recently, we reported on SPCE with 20 nm aluminum, ¹⁰ where we studied 2-aminopurine, which emits between 350 and 450 nm. In this manuscript we describe ultraviolet SPCE of 2,5-diphenyl-1,3,4-oxadiazole (PPD) deposited on an aluminized quartz slide. The PPD emission between 300 and 400 nm with a maximum of about 350 nm mimics tryptophan fluorescence. The possibility of accessing the UV region may enable detection of unlabeled proteins using SPCE.

Materials and Methods

Reflectance Calculations. SPCE is closely related (a reverse process) to SPR. The angular distribution of the radiated light in the glass/quartz prism is determined by the same wavevector matching requirements as for SPR. For this reason the equations used in SPR theory can be used to describe the angular distribution of SPCE. Of course, the SPCE-related calculations should be done for emission wavelength, as has been clarified in the recent theoretical treatment of SPCE.¹¹ The equations needed to calculate reflectivities of the thin films can be found in the literature. 12,13 These calculations can also be performed (up to four phases) using web-based software. 14 In this manuscript the reflectance profiles of the thin film 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 excitation and/or emission wavelength.

Sample Preparation. Quartz slides were coated by vapor deposition by EMF Corp. (Ithaca, NY). A 20 nm thick layer of aluminum was deposited on the quartz followed by a 10 nm SiO_2 protective layer. This relatively thick silica layer has a dual function. First, it protects the aluminum film and slows

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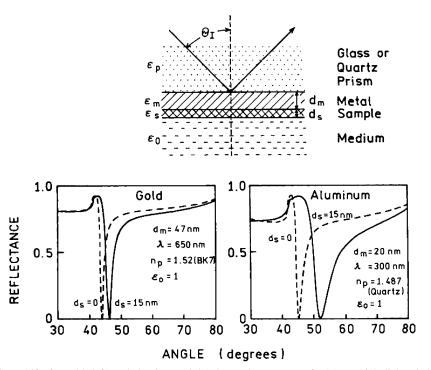


Figure 1. Comparison of SPR shifts for gold (left) and aluminum (right) due to the presence of a 15 nm thick dielectric layer with refractive index $n_s = 1.5$. (Top) Four-layer system used for shifts calculation. The dielectric constants were $\epsilon_{Au}^{650} = -11.55 + 1.08i$ and $\epsilon_{Al}^{300} = -11.03 + 1.67i$.

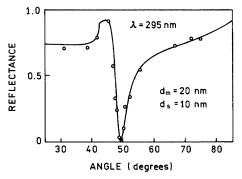


Figure 2. Calculated (solid line) and measured (open circles) reflectances of the 295 nm line from a frequency-doubled rhodamine 6 G dye laser for a quartz slide coated with 20 nm of aluminum (d_m) and 10 nm of SiO_2 (d_s). The dielectric constant for aluminum at 295 nm was $\epsilon_{Al}^{295} = -10.67 + 1.60i$.

down the oxidation process. Second, the fluorophores are deposited at or more than 100 Å from the aluminum surface, reducing the possibility of quenching by the metal. Fluorophores were deposited on the surface by spin coating at 3000 rpm 0.2% solution of poly(methyl methacrylate) (PMMA, M_w 15 000; Aldrich) in toluene/ethanol (1:1, v/v). The PMMA solutions contained 2,5-diphenyl-1,3,4-oxadiazole (PPD, 1.5×10^{-3} M) from Sigma. The reference slide for the control experiment was prepared from identical 0.2% PMMA solution without PPD.

We examined the reflectances of our aluminized slides before sample deposition. The measured reflectance minimum appears at 49° (Figure 2, open circles). This value is about 4° higher than a minimum calculated for bare 20 nm aluminum film (Figure 1, right). This 4° shift is consistent with the 10 nm SiO₂ protective layer on the top of the metal (Figure 2, solid line). This confirmed that the slides were prepared precisely as assumed, with 20 nm of aluminum and 10 nm of SiO₂.

Fluorescence Measurements. The spin-coated slides were attached to a hemicylindrical prism made of quartz using nonfluorescent index matching fluid (glycerol). This combined sample was positioned on a precise rotary stage which allows

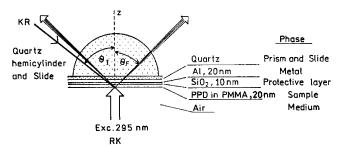


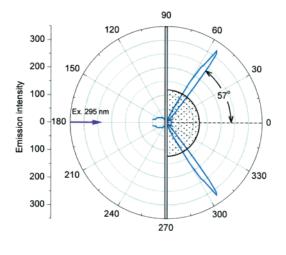
Figure 3. Schematic of sample-coupling prism configuration for SPCE fluorescence of PPD. The sample can be excited directly (RK configuration) or through the prism at θ_I angle (KR configuration). SPCE radiation appears as a hollow cone of p-polarized light.

excitation and observation at any desired angle relative to the vertical axis around the cylinder.⁶ For excitation we used mainly the reverse Kretschmann (RK) configuration (Figure 3). 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 with the incident light. The angle of incidence does not matter, but we used normal incidence.

Observation of the angular distribution of the emission was performed with a 3 mm diameter UV-transmitting fiber covered with a 500 µm vertical slit, positioned about 15 cm from the sample. This corresponds to an acceptance angle below 0.2°. The output of fiber was directed to a 8000 SLM spectrofluorometer or a 10-GHz frequency domain fluorometer.¹⁵

For excitation we used the second harmonic (295 nm) from a Rhodamine 6G dye laser. The dye laser was pumped by a mode-locked argon ion laser, 76 MHz repetition rate, 120 ps half-width, and cavity dumped at 3.8 MHz repetition rate. Scattered light at 295 nm was suppressed by observation through a 0-54 Corning cutoff filter supported for intensity and lifetime measurements with a 350 nm interference filter.

The multifrequency phase and modulation data were analyzed in terms of the multiexponential model, as described previously. 16 The multiexponential model is used to describe the form



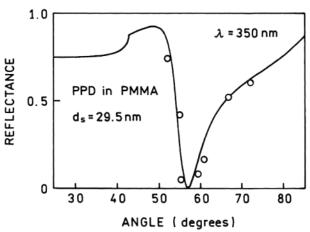


Figure 4. (Top) Angular distribution of PPD SPCE. (Bottom) Calculated (solid line) and measured (open circles) reflectances at the 350 nm line from pyridine 1 dye laser. Calculations were done for combined thickness of the SiO₂ protective layer (10 nm) and PPD-doped PMMA layer (19.5 nm) with refractive index $n_s = 1.5$. The dielectric constant for aluminum was $\epsilon_{\rm Al}^{350} = -15.24 + 2.55i$.

of the intensity decay. We are not assigning molecular significance to the recovered parameters.

Results

Although aluminum has already been suggested for use in SPR,¹⁷ we are not aware of any experimental reports on ultraviolet SPCE using aluminum films. The use of aluminum with fluorescence was not obvious because of observed strong quenching on aluminum surfaces.¹⁸ To avoid the quenching effects we used a 10 nm protective layer of SiO₂. This protective layer, added in the vapor deposition procedure, also protected the surface from oxidation.

The angle-dependent intensities are shown in Figure 4, top. The 295 nm excitation was used in RK mode, in which the sample was excited directly from the air. It should be noted that in the RK configuration the surface plasmons are not directly induced by the excitation beam. The directional emission is an effect of interactions of excited fluorophores with surface plasmons. The SPCE of PPD (emission maximum = 350 nm) is sharply distributed around 57° from the normal axis. The free-space (FS) emission to the air (angles 90–270° in Figure 4, top) is very weak compared to SPCE. The integrated intensities show that the ratio of SPCE/FS intensity is about 1.66. This indicates a very high coupling efficiency of excited PPD dipoles to the surface plasmons, about 62%.

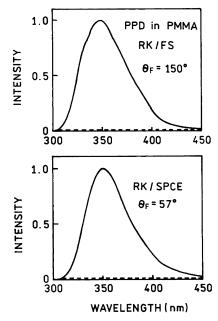


Figure 5. (Top) Fluorescence spectrum of PPD spin-coated on aluminized quartz slide observed in free space with reverse Kretschmann configuration (RK/FS). (Bottom) SPCE spectrum observed through the coupling prism (RK/SPCE). The angles are determined in Figure 4, top. The dashed lines are signals recorded for reference slide without PPD.

The observed SPCE of PPD was almost completely p-polarized, irrespective of the polarization of the excitation beam. The possibility of using s-polarization of excitation with simultaneous detection of p-polarized emission (crossed polarizers) provides an additional possibility for rejection of scattered excitation and polarized background.

We also measured the reflectance of a 350 nm laser beam (from a pyridine 1 dye laser) from the sample slide (Figure 4, bottom, open circles). The minimum of reflectance was found to be between 55° and 60°, in good agreement with SPCE direction. The calculated angular dependence of reflectance (Figure 4, bottom, solid line) reaches a minimum at 57° for a sample thickness of about 30 nm. The calculations were done for the five-phase system shown in Figure 3.

The emission spectrum of SPCE (Figure 5, bottom) is characteristic for PPD fluorescence and similar to the free-space spectrum (Figure 5, top). The fluorescence background signal, from an identically prepared slide without PPD, is negligible for both free-space emission and SPCE (Figure 5, dashed lines). The spectra in Figure 5 were recorded with a vertically oriented polarizer on excitation and a horizontally oriented polarizer on observation.

The SPCE signal was over 25-fold lower for a vertically oriented polarizer on observation. The high p-polarization of the observed directional emission (P > 0.95) proves its origin with surface plasmons and not free-space emission of PPD. The free-space fluorescence of PPD was 2-3-fold stronger for the vertically oriented polarizer on observation, as expected for the photoselection.

The dielectric properties of metals strongly depend on the wavelength of the light. In SPR analysis it is well known that reflectivity minimum is wavelength dependent, ^{2,19,20} shifting responses toward smaller angles for longer wavelengths. We recorded SPCE spectra at different angles of observation (Figure 6). The normalized spectra in Figure 6 show a remarkably large shift for a few degree change in observation. The dispersive

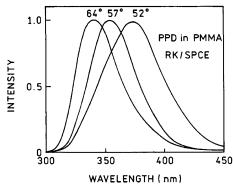


Figure 6. Observation angle dependent SPCE spectra of PPD spin coated from PMMA solution on aluminized quartz slide. The sample was excited directly (295 nm) in reverse Kretschmann configuration (RK/SPCE).

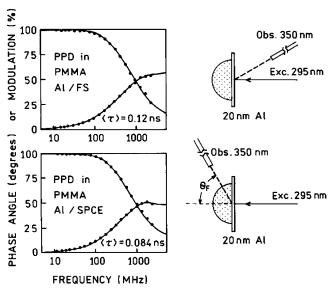


Figure 7. Frequency-domain intensity decays of PPD spin coated from a solution of PMMA on an aluminized quartz slide measured with RK/ FS (top) and RK/SPCE (bottom) configuration.

TABLE 1: Multiexponential Analysis of PPD in PMMA Intensity Decays. PPD in 0.2% PMMA Was Spin Coated on Aluminized (Al) and Unaluminized (Q) Quartz Slides (excitation = 295 nm, observation = 350 nm, RK configuration)

conditions	$\langle \tau \rangle$ (ns)	$\bar{\tau}$ (ns)	α_1	τ_1 (ns)	α_2			τ ₃ (ns)	
Q, FS Al, FS Al, SPCE	0.12	0.35	0.658	0.02	0.370 0.268 0.190	0.22	0.074	0.61	2.4

 $^{a}\langle \tau \rangle = \sum_{i} \alpha_{i} \tau_{i}$. $^{b} \bar{\tau} = \sum_{i} f_{i} \tau_{i}$, $f_{i} = \alpha_{i} \tau_{i} / \sum_{i} \alpha_{i} \tau_{i}$.

properties of SPCE do not require any additional optical components.

In our earlier studies on metal-enhanced fluorescence²¹⁻²⁴ we found significant decreases in lifetimes when fluorophores were near metallic particles. Hence, we examined the lifetime of PPD (Figure 7). Compared to the free-space fluorescence (Figure 7, top), the lifetime of PPD SPCE is shortened by about 30%, which we consider a modest change only. These lifetimes, measured at RK/FS and RK/SPCE configurations (Figure 7), are about 4-fold shorter than the lifetime of PPD in PMMA spin coated on the quartz slide without metal (Table 1). At this time we do not fully understand how the surface plasmons and fluorophore molecules interact to determine the SPCE intensity decays.

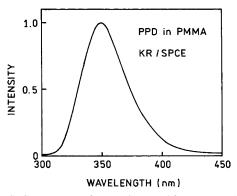


Figure 8. SPCE spectrum of PPD spin coated from a PMMA solution on an aluminized quartz slide. The excitation was with Kretschmann configuration through the prism (Figure 3). The KR/SPCE excitation is many-fold stronger than direct (RK) excitation, and attention must be paid to the photobleaching.

Finally, we used the Kretschmann configuration (Figure 3) with an excitation (SPR) angle of about 60° with a horizontally oriented polarizer on excitation. The emission spectrum at 57° observation measured in this configuration (KR/SPCE) is shown in Figure 8. The intensity of the PPD emission, also highly p-polarized in the KR/SPCE configuration, was approximately 20-fold higher than that in the RK/SPCE setup with the same excitation power. In the case of the Kretschmann configuration, the excitation is provided by an evanescent field of surface plasmon wave, which can be many fold stronger than the incident light. Such a strong excitation caused strong photobleaching of usually highly photostable PPD. We had to significantly attenuate the excitation intensity in order to record the emission spectrum in the KR/SPCE configuration.

Discussion

The measurements presented in this manuscript demonstrate the possibility of using SPCE with UV wavelengths for excitation and emission. UV fluorophores are routinely used to label proteins and membranes. Also, intrinsic emission from proteins between 300 and 450 nm can be studied with aluminum-coated slides.

The 100 Å SiO₂ protective layers are surprisingly stable. We washed the used slides with toluene/ethanol mixture and then spin coated again with the PPD-doped PMMA solution as described in the Materials and Methods section. The intensity and SPCE angle (57°) observed in the RK configuration were similar to those of the original slide. We concluded that the slides with the 100 Å SiO₂ protective layer on the top of aluminum can be easily reused. We already noticed that aluminized slides with 100 Å SiO₂ layers are resistant to the water solution. 10 We could not reuse silvered slides with 50 Å SiO₂ layers in our previous SPCE studies.^{6–8}

In earlier reports we^{7,8} and others²⁵ demonstrated the usefulness of SPCE techniques for biochemical assays which utilize surface-localized chemistry. Our presented results with aluminum show that the range of wavelengths can be extended down to the ultraviolet region around 300 nm.

The directionality and polarization properties of SPCE provide an opportunity to reduce the unwanted background. In the UV region the background is often a limitation factor. We believe that many previously impossible UV experiments can now be done with SPCE. In particular, the Kretschmann (KR) configuration (Figure 3) provides excitation through an evanescent field near the metal surface, which further reduces the background from the bulk solution and enables the SPCE measurements in optically dense media.^{6,7} In the KR configuration (Figure 8) we were able to reduce the excitation about 30-fold in order to obtain similar a SPCE as with the RK configuration.

Finally, the dispersive properties of SPCE provide a unique opportunity for construction of miniaturized spectrofluorometers with a minimum of optical components.

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