

Surface Plasmon Coupled Fluorescence in the Ultraviolet and Visible Spectral Regions Using Zinc Thin Films

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The use of zinc thin films deposited onto glass supports for surface plasmon coupled fluorescence (SPCF) over a broad 200 nm wavelength range is demonstrated. Fresnel calculations performed in the ultraviolet and visible spectral range are predicted to generate surface plasmon modes in 30 nm zinc thin films. In this spectral range, the extent of coupling of light to zinc thin films was shown to be significant as compared to similar aluminum, gold, and silver thin films. The experimental demonstration of SPCF using 30 nm zinc thin films in the ultraviolet and visible spectral regions was undertaken using three different fluorophores 2-AP, POPOP, and FITC, respectively. Surface plasmon coupled fluorescence from zinc thin films was *p*-polarized and highly directional with λ_{max} conferred at an angle of 58, 68, and 60° for FITC, POPOP, and 2-AP, respectively. *s*-Polarized emission from zinc thin films was negligible for all fluorophores except for a sample spin coated from a 10% PVA solution, which resulted in significant *s*-polarized emission due to the generation of waveguide modes. The experimental results are consistent with reflectivity curves that are theoretically predicted using Fresnel calculations. Given the growing use and utility of plasmon-enhanced fluorescence in the analytical and biological sciences, our findings will serve as a useful tool for workers in the ultraviolet and visible spectral regions.

Since the early observations that the spontaneous emission rate of fluorescent species can be modified with close-proximity planar metal surfaces,^{1–5} numerous studies have focused on metal–fluorophore interactions.^{6–8} The spontaneous emission of fluorescent species near-to metals follows a distance dependent radiative and/or nonradiative decay channel, which is affected by

the dipole orientation of the fluorescent species.⁹ The emission rate oscillates as the distance between the metal and the dipole is steadily increased, and the strength of these oscillations decreases since the dipole is a point source. A parallel dipole (*s*-polarized) is canceled out by its own image on the metal surface while a perpendicular dipole (*p*-polarized) is coupled to the surface plasmon modes and is subsequently enhanced. Several authors have calculated the distance dependent emission rate with the assumption that the metal surface is perfect and the rotation of the dipole moment is within the excited-state lifetime.¹⁰

When the distance between the metal surface and the fluorescent species is larger than 300 nm, decay processes occur through traditional far-field radiation (i.e., fluorescence), while excited states can couple/induce surface plasmons at ≈ 10 –300 nm. Below 10 nm, fluorescence typically decays through nonradiative channels, often referred to as fluorescence damping or quenching.¹¹ The coupling of fluorescence from a population of randomly oriented fluorescent species and the subsequent polarized and directional emission from the metal surface is well described by a phenomenon called surface plasmon coupled fluorescence.^{8,12,13} As a result, it became possible to achieve better detectability of fluorophores at metal interfaces, which facilitates the detection of surface bound biomolecules with a technique often referred to as surface plasmon fluorescence spectroscopy (SPFS).¹³

In SPFS, the excitation of the fluorescent species can be achieved either directly from the air side (reverse Kretschmann configuration) or through a prism (Kretschmann configuration).¹³ In the reverse Kretschmann configuration, the fluorescent species are excited from the air side (at any incidence angle) and the coupled fluorescence emission is then detected from the prism side. This configuration also allows the detection of isotropic (free-space) emission from the air side. In the Kretschmann configuration, the *p*-polarized light emerges through the prism at the surface plasmon resonance angle and generates surface plasmon modes at the metal–dielectric interface. The generated evanescent wave penetrates the subsequent dielectric layers, and the excitation of the fluorescent species is achieved. The fluorescence emission is then detected from the air side as free-space emission or from the prism side, where the surface plasmon coupled

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fluorescence is coupled at a specific angle (cone). Both configurations are highly attractive in analytical sensing applications.^{14,15}

In previous studies on SPCF, aluminum thin films were demonstrated to have use in the UV spectral range¹⁶ and silver thin films were mostly used in the visible spectral range.¹⁷ Gold¹⁷ and copper¹⁸ thin films, which have similar optical and electronic properties, have found use in the red spectral region. The use of these noble metals in SPFS is due to their nearly free-electron behavior in these spectral regions, giving rise to a low ϵ_i/ϵ_r ratio (ϵ_i , imaginary component of the dielectric function; ϵ_r , real component of the dielectric function), which in turn yields sharp, narrow, and deep reflectivity minima. Planar gold films are usually the substrate of choice^{13,17} due to their versatility, i.e., they are inert and can undergo further chemical modification without losing physical and electronic properties. The optimum thickness of the metals for SPCF was previously determined to be ≈ 20 nm for aluminum thin films,¹⁶ 35 nm for copper thin films¹⁸ to ≈ 50 nm for gold thin films¹⁷ and silver thin films.¹⁹

One can find numerous reports on the utilization of SPCF in bioassays.^{20–25} These include studies for DNA hybridization,^{20,21,24} immunoassays,^{25–28} and protein detection.¹⁴ In all of these reports, one of the biological assay components is covalently linked to the metal surface and their interactions with the biomolecule of interest in the presence of a third fluorophore-labeled biomolecule is monitored via the change in SPCF intensity from the back of the prism. Subsequently, the concentration of the biomolecule of interest is directly calculated from the SPCF intensity.^{14,15}

Zinc has been rarely used in combination with fluorescence;^{29,30} however, considerable attention has been given to zinc due to other settings to its wide bandgap (3.37 eV) and large exciton binding energy (60 meV) at room temperature.³¹ Zinc has a hexagonal crystal structure^{32,33} and readily forms an oxide. In

addition, the imaginary component (ϵ_i) of the dielectric function for zinc is very large in the visible region of the spectrum with peaks at 1.6 and 0.8 eV readily arising from the band transitions.³³ This results in a high ϵ_i/ϵ_r (as compared to the noble metals) and broad and shallow surface plasmon resonances, which can readily be calculated.

In this work, the applicability of zinc thin films in combination with fluorescence to surface plasmon fluorescence spectroscopy is demonstrated both theoretically and experimentally. Theoretical Fresnel calculations were employed to determine the optimum thickness for the generation of surface plasmon modes in zinc thin films. Fresnel calculations were also used in the investigation of the spectral range where the surface plasmons in zinc can effectively be generated as well as in direct comparison with aluminum, gold, and silver films. In this regard, to demonstrate the applicability of zinc thin films for use in SPFS, 30 nm zinc thin films with a 10 nm SiO₂ overlayer were thermally evaporated onto glass supports that have optical transmission above 365 nm. Surface plasmon coupled and free-space fluorescence from fluorophores with emission in the ultraviolet and visible spectral regions were measured using the reverse Kretschmann configuration. The theoretical calculations and experimental data are in very good agreement. Finally, we place our findings in context with immunoassays and analytical based sensing, where the penetration depth into optically dense media can be both modeled and tuned using different metallic thin film sensing supports.

EXPERIMENTAL SECTION

Materials. All fluorophores, 2-aminopurine (2-AP), 1,4-bis(5-phenyl-2-oxazolyl)benzene (POPOP), fluorescein isothiocyanate (FITC), poly(vinyl)alcohol (PVA, 98% hydrolyzed 13 000–23 000 MW), poly(methyl methacrylate) (PMMA, 100 000 MW), chloroform (99.8% ACS Reagent) and silane-prep glass microscope slides were purchased from Sigma-Aldrich chemical company (Milwaukee, WI). Thirty nanometers thick zinc thin films with 10 nm thick SiO₂ overlayer were deposited onto silane-prep glass microscope slides by Thin Films, Inc., Hillsborough, NJ.

Sample Preparation. Fluorophores were deposited onto zinc thin films by spin coating a solution of polymers (PVA in water or PMMA in chloroform) containing the fluorophores. Stock solutions of FITC (1 mM) and 2-AP (3.5 mM) were prepared in deionized water and then diluted with various solutions of PVA that were prepared in water. The final concentrations of fluorophore/polymer solutions used to spin coat zinc thin films were as follows: 0.1 mM FITC in 0.1, 1, and 10% PVA and 1 mM 2-AP in 5% PVA. A stock solution of POPOP (1 mM) was prepared in chloroform and was mixed with a 10% PMMA solution to make a POPOP solution with the following final concentrations: 0.1 mM POPOP in 5% PMMA. A volume of 40 μ L of fluorophore/polymer solutions were spin-coated onto zinc thin films (1 cm $\times$ 1 cm) using a Chemat Technology spin coater (model KW-4A) with the following speeds: setting 1, 9 s; setting 2, 20 s. The thickness of the polymer films was measured using a Molecular Imaging Picoplus atomic force microscope at a scan rate of 1 Hz with 512 $\times$ 512 pixel resolution in the tapping mode, and the average film thickness was determined to be 25, 70, and 400 nm for the 0.1, 5, and 10% PVA films and 750 nm for the 5% PMMA film (Supporting Information, Figure S2).

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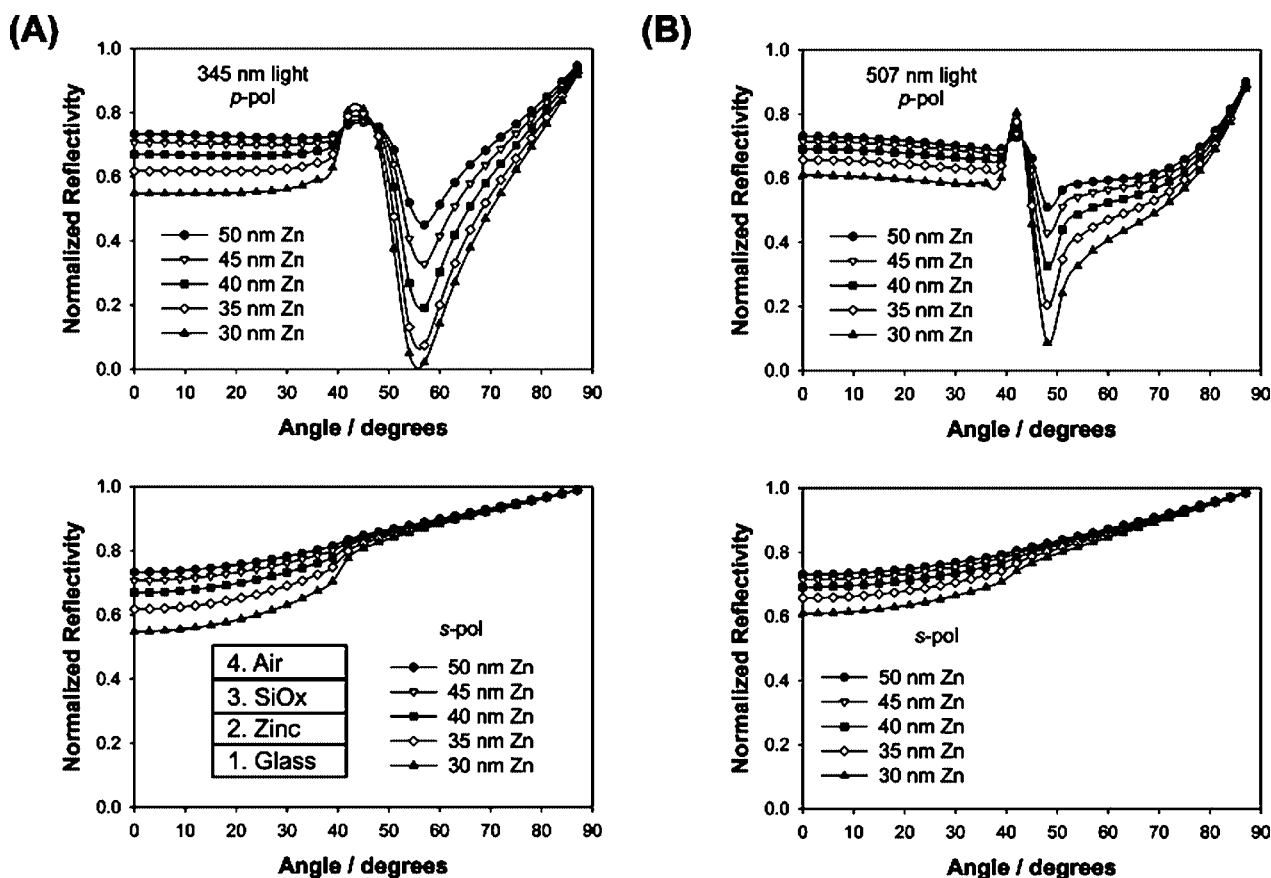


Figure 1. Determination of the thickness of zinc substrates for surface plasmon coupled fluorescence. Four-phase Fresnel reflectivity curves of *p*- (top) and *s*- (bottom) polarized light at (A) 345 and (B) 507 nm for various thicknesses of zinc substrates with a 10 nm SiO_x overlayer.

It is important to note that since the thickness of the polymer film spin coated onto the metal films is dependent on the size of the support, the type, and the settings of the spin coater itself, similar solution preparation conditions and settings were used to reproduce the results presented in this study.

Surface Plasmon Fluorescence Spectroscopy (SPFS).

SPFS measurements were made as follows: the fluorophore/polymer-coated zinc thin films were attached to a right-angle prism made of BK7 glass with index matching fluid. This combined sample was positioned on a precise rotary stage (*x*-*z*) that allows excitation and observation at any desired angle relative to the vertical axis (*z*-axis) along the prism. The sample was excited using the reverse Kretschmann configuration from the air or sample side, which has a refractive index lower than the prism. The excitation of 2-AP and FITC was carried out with a laser (335 and 473 nm, respectively) at an angle of 90°. The excitation of POPOP was from a UV light source (Mikropack D-2000 deuterium) that was collimated to a 5 mm spot on the sample geometry at an angle of 90°.

Observation of the surface plasmon coupled and free-space emission were performed with a 600 μ m diameter fiber bundle, covered with a 200 μ m vertical slit, and positioned about 15 cm from the sample. This corresponds to an acceptance angle below 0.1°. The output of the fiber was connected to an Ocean Optics HD2000 spectrofluorometer to measure the fluorescence emission spectra through a 400 nm long-pass filter for 2-AP and POPOP and a 488 nm super notch filter (Semrock) for FITC.

Real-color photographs of the surface plasmon coupled emission were taken through an emission filter used for the excitation of the samples placed on a hemispherical prism.

Theory and Fresnel Calculations. It is well-known that surface plasmon modes in metallic thin films can be generated by fluorescent species in close proximity, i.e., in the near-field.⁹ The optical properties of metals can be predicted using the Drude dispersion model.³⁴ The coupling of light emission from fluorescent species to metal surfaces depends on the matching of the wavevector of the incident light (k_0) with the wavevector of the surface plasmons (k_{sp}) according to the following equation^{13,35}

$$k_{sp} = k_0 \left(\frac{\epsilon_m \epsilon_s}{\epsilon_m + \epsilon_s} \right)^{1/2} \quad (1)$$

where ϵ_m and ϵ_s are the real parts of the dielectric constants of the metal ($\epsilon_m = \epsilon_r + \epsilon_i$) and the sample ($\epsilon_s = \epsilon_r + \epsilon_i$) above the metal film, respectively. The conditions for SPR excitation are met when the following condition is satisfied

$$k_{sp} = k_x = k_0 n_p \sin \theta_{sp} \quad (2)$$

where n_p is the refractive index of the prism, θ_{sp} is the surface plasmon angle, k_x and k_0 are the wavevectors in the metal (*x*-component) and free space (incident light), respectively. From eq 2, it follows that the reflectivity and transmissivity of incident light at the metal–dielectric interface are determined by θ_{sp} . The reflectivity at the surface below the surface plasmon coupling angle, θ_{sp} , is very high due to the presence of an evaporated metal layer that acts as a mirror and reflects most of the transmitted light.¹³ At just above θ_{sp} , the metal surface acts as a resonator for

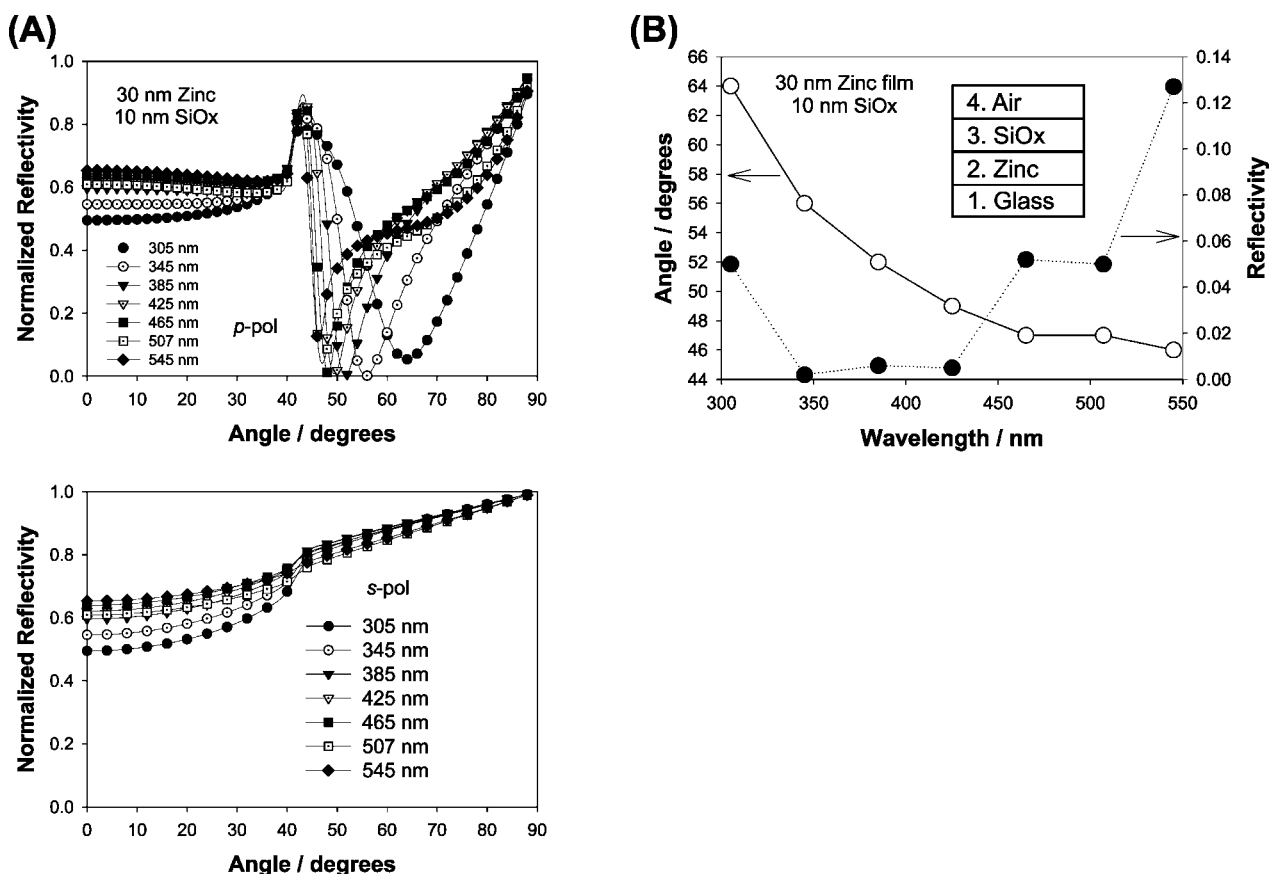


Figure 2. Determination of the wavelength range for surface plasmon coupled fluorescence from zinc substrates. Four-phase Fresnel reflectivity curves of (A) *p*- (top) and *s*- (bottom) polarized light at 305, 345, 385, 425, 465, 507, and 545 nm for 30 nm thick zinc substrates with a 10 nm SiO_x overlayer and (B) a plot of reflectivity minimum angles versus wavelength of incident light for 30 nm zinc substrates with a 10 nm SiO_x overlayer.

incident light and gives rise to the resonant excitation of a surface plasmon, which reradiates according to the dispersion curve for surface plasmons.¹³ Fresnel calculations (using a macro procedure written for Igor Pro software) were performed to account for each the different optical properties of each dielectric layer and their respective thicknesses, surface plasmon resonance conditions.

Penetration depth calculations were performed for metals using three-phase (glass/metal/water) Fresnel calculations. In this regard, the maximum value for the *z*-component of the electric field (E_z^2) that occurs at the angle of reflectivity minimum is normalized with respect to the highest value and plotted against the thickness (depth) above the metal.³⁶

RESULTS AND DISCUSSION

Since zinc has a band gap energy of 3.37 eV (368 nm) at room temperature and finite-difference time-domain (FDTD) calculations predict the occurrence of a surface plasmon resonance (SPR) peak and increased electric fields around zinc nanostructures at 380 nm, one can expect that surface plasmon modes of zinc can be generated by *p*-polarized light or near-field fluorescent species emitting at these wavelengths. In this regard, Fresnel calculations for two wavelengths of light (1) overlapping (345 nm) and (2) red-shifted (507 nm) with respect to the SPR peak of zinc were performed to determine the ideal thickness for the zinc thin

films for SPCF. Figure 1 shows the four-phase Fresnel reflectivity curves of 345 and 507 nm *p*- and *s*-polarized light for various thicknesses of zinc thin films and with a 10 nm SiO_x overlayer. SiO_x is commonly used as a protective layer and affords for silane chemistry to be carried out on metallic films. Fresnel calculations show, in both cases, that a reflectivity curve minimum occurs for 30 nm thick zinc films, and thus the optimum thickness with zinc thin films is 30 nm. The calculated optimum thickness for zinc thin films is in the same range of thicknesses reported for other metallic thin films employed in SPFS.^{16–18} The angle of the reflectivity minimum, which varies with wavelength, occurs at 56 and 47° for 345 and 507 nm *p*-polarized light, respectively. As shown before,¹⁸ *s*-polarized light incident on metallic thin films does not couple and induce surface plasmons, which is found to hold for zinc thin films (Figure 1A,B,bottom).

To determine the range of fluorophores that will most efficiently couple to zinc thin films, four-phase Fresnel reflectivity curves for multiple wavelengths were calculated and are shown in Figure 2. The wavelength range, 305–545 nm, was chosen based on the predicted occurrence of an SPR peak at and around 380 nm. Figure 2A shows that *p*-polarized light over a 200 nm wavelength range (305–545 nm) generates surface plasmon modes of 30 nm zinc thin films with a 10 nm SiO_x overlayer, while *s*-polarized light does not couple to surface plasmons. It is important to note that Fresnel reflectivity calculations predict that light at longer wavelengths (<550 nm) does not efficiently generate surface plasmon modes of zinc

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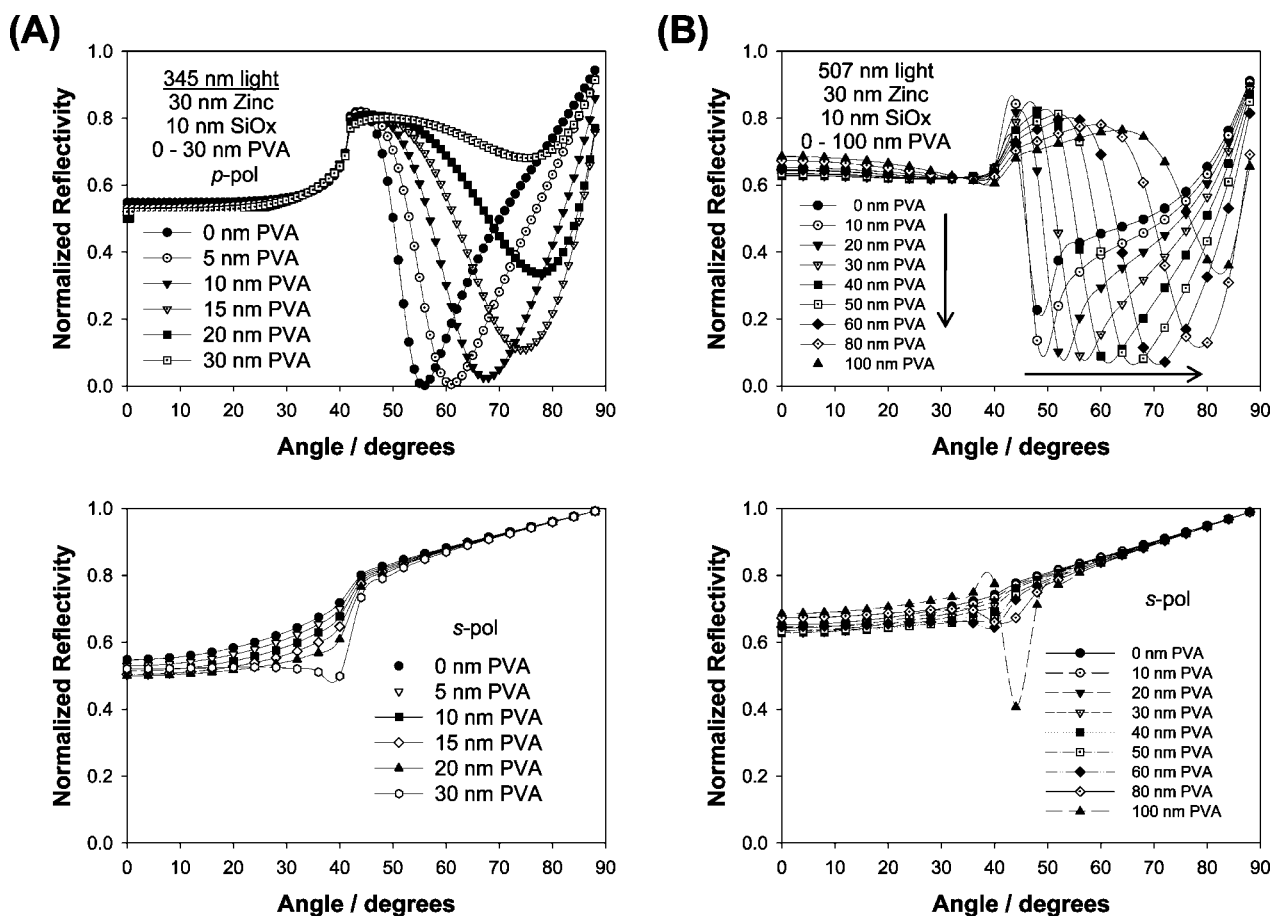


Figure 3. Optimization of 30 nm zinc substrates with polymer overlayer for surface plasmon coupled fluorescence. Five-phase Fresnel reflectivity curves of *p*- (top) and *s*- (bottom) polarized light at (A) 345 and (B) 507 nm for 30 nm thick zinc substrates with various thicknesses of a PVA overlayer.

(data not shown). Figure 2B shows the plot of normalized reflectivity and angles of the reflectivity minimum with respect to the wavelength of light. The angle of the reflectivity minimum occurs at 64° for *p*-polarized light at 305 nm and decreases as the wavelength is increased. Since the normalized reflectivity is less than 5% in the wavelength range of 305–507 nm (Figure 2B), fluorophore emission in this wavelength range is predicted to most effectively couple to zinc thin films.

In addition to the thickness of the metal film, surface plasmon coupled emission is also influenced by the thickness of the sample containing the fluorophores.¹⁹ In a typical fluorescence-based bioassay (a sandwich immunoassay) constructed on a surface support, the fluorophores can be located as far as 30 nm from the surface. To determine the effect of sample thickness on the optical response from zinc thin films, five-phase Fresnel reflectivity curves for light at 345 and 507 nm, for 30 nm thick zinc thin films, 10 nm SiO_x overlayer, and various thicknesses of PVA were calculated, Figure 3. These wavelengths are chosen to emulate the typical emission wavelengths of commonly used fluorophores used in bioassays, namely, 2-AP and fluorescein. Figure 3A shows that surface plasmons can efficiently be generated only by *p*-polarized emission at 345 nm from a sample up to 25 nm in thickness (10 nm SiO_x + 15 nm PVA). The degree of coupling of *p*-polarized light (or fluorescence emission) decreases and becomes inefficient for >40 nm thick samples. The Fresnel calculations also predict a 5° shift in angle of the reflectivity minimum

for every 5 nm increase in sample thickness. Figure 3B shows that the generation of surface plasmons by *p*-polarized light at 507 nm is efficient up to 90 nm (10 nm SiO_x + 80 nm PVA). A shift and broadening in the reflectivity minimum, as a result of an increase in sample thickness, for the coupling of *p*-polarized light at 507 nm is also predicted. Figure 3 also shows that *s*-polarized light both at 345 and 507 nm is not predicted to generate or couple to surface plasmons of zinc thin films with a SiO_x overlayer and the various PVA thicknesses.

The optimum thickness of metals other than zinc used in SPFS was previously determined to be 20 nm for aluminum thin films,¹⁶ 35 nm for copper thin films,¹⁸ to 50 nm for gold thin films¹⁷ and silver thin films.¹⁹ To demonstrate the advantages of using zinc thin films over aluminum, gold, and silver thin films in SPFS, five-phase Fresnel reflectivity curves for light at 305–517 nm were calculated and compared for these metals, cf. Figures 4 and 5. Figure 4 shows the comparison of Fresnel reflectivity curves for zinc (30 nm) and aluminum (20 nm)¹⁶ thin films with 10 nm SiO_x and 12 nm PVA overlayers. These overlayer thicknesses (22 nm) were used in a previous study on SPFS¹⁶ and also encompasses the range of distances of fluorophores from the metal surface used in SPCF-based bioassays. These reflectivity curves show that only *p*-polarized light efficiently generates surface plasmon modes of zinc and aluminum thin films in the 345–517 and 305–517 nm spectral range, respectively. Two major differences in the reflectivity curves for zinc and aluminum thin films can be observed:

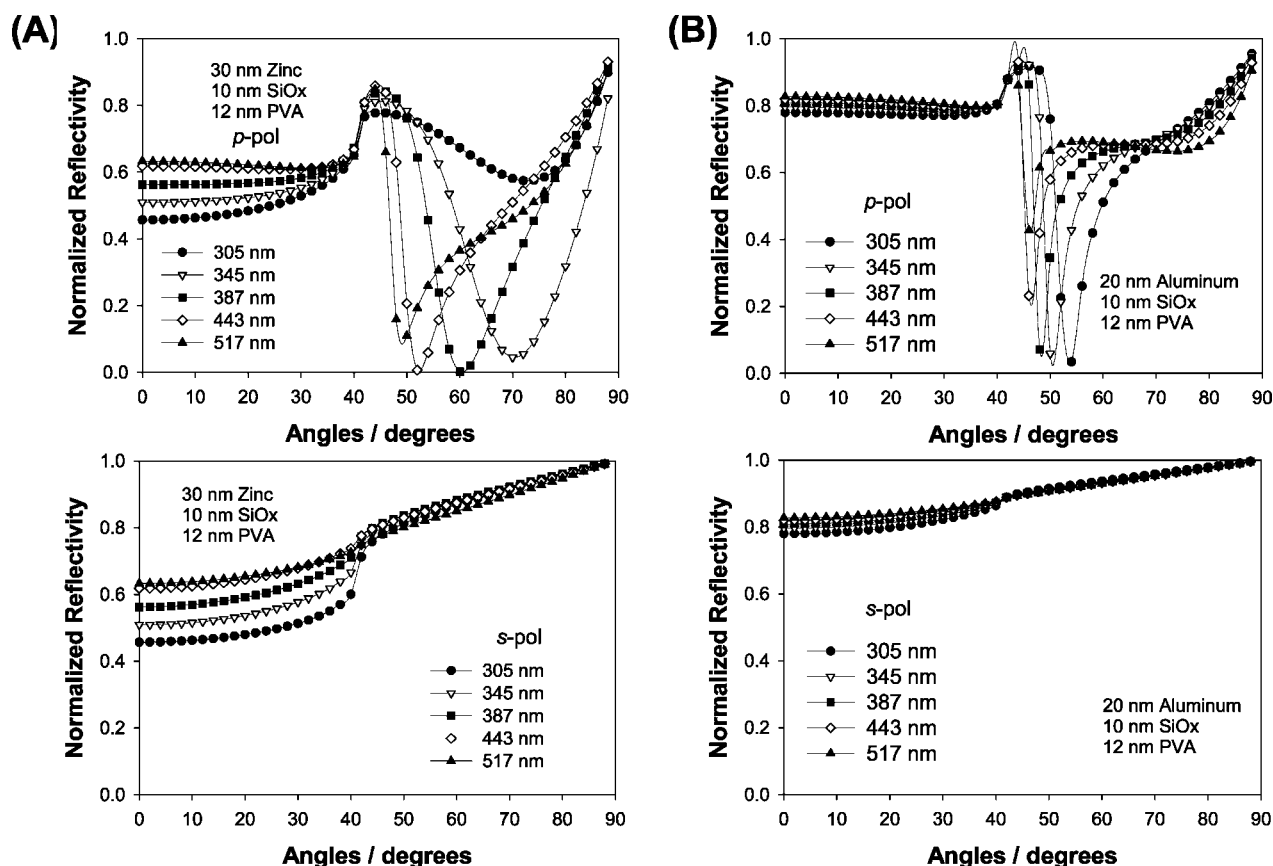


Figure 4. Direct comparison of wavelength-dependent reflectivity curves between zinc and aluminum substrates for surface plasmon fluorescence spectroscopy. Five-phase Fresnel reflectivity curves of *p*- (top) and *s*- (bottom) polarized light at 305, 345, 387, 443, and 517 nm for (A) 30 nm thick zinc substrates and (B) 20 nm thick aluminum substrates with two subsequent overlayers of 10 nm SiO_x and 12 nm PVA.

(1) the reflectivity curves for zinc appear broader than the reflectivity curves for aluminum and (2) the efficiency of generation of surface plasmon modes is significantly better at wavelengths >443 nm. The observations imply that *p*-polarized fluorescence emission over a broader range of wavelengths is predicted to couple to zinc thin films as compared to aluminum thin films. This is seen as a notable finding for immunoassays given the widespread availability of different color fluorophores and fluorescent proteins.

To further assess the versatility of zinc thin films, the reflectivity curves of light at 335–517 nm for zinc films were compared with the reflectivity curves for gold and silver thin films with the same overlayer thicknesses, cf. Figure 5. While the reflectivity curves for silver thin films show the generation of surface plasmon modes at 443 and 517 nm (at a lesser extent than both zinc and aluminum thin films), the reflectivity curves for gold thin films are predicted not to generate surface plasmon modes with the 22 nm thick overlayer. Since most of the commercial bioassays employ fluorophores (and some quantum dots) emitting in the longer wavelengths and reliable and low-cost excitation sources are commonly available in the visible range, zinc thin films are predicted to be the better single assay platform in SPFS for use both in the UV and visible spectral range than other metallic thin films.

The Fresnel reflectivity curves predict that *p*-polarized light in the 345–545 nm spectral regions can generate surface plasmon modes and efficiently couple to 30 nm thick zinc thin films with up to a 90 nm thick overlayer. These theoretical predictions were subsequently confirmed with experimental data as explained in

the following sections. Figure 6A shows the experimental geometry used for SPFS studies. In this regard, 30 nm thick zinc thin films and a subsequent 10 nm SiO_x overlayer were thermally evaporated onto glass supports. Fluorophores were mixed with a polymer solution (either PVA or PMMA) and spin-coated onto the zinc thin films. Fluorophore-coated zinc thin films were then attached to a hemispherical prism and surface plasmon coupled fluorescence and free-space emission were measured after the excitation of the fluorophores from the air side (reverse Kretschmann configuration). The SPCF is measured at 525 nm from the prism side and appears as a green ring due to the symmetry considerations as seen for a FITC sample shown in Figure 6A.

Figure 6B shows the normalized *p*- and *s*-polarized fluorescence intensity from 50 mM FITC in PVA films deposited onto zinc thin films. The initial concentration of PVA stock solutions is varied between 0.1 and 10% (the final concentration of FITC was the same in all solutions) to study the effect of sample thickness on SPCF emission from zinc thin films. The thickness of the PVA film deposited onto zinc thin films is known to increase as the initial concentration of the stock solution is increased.¹⁹ The SPCF emission from FITC-coated zinc thin films is measured at angles of 0–90°, and the free-space emission is measured at 125°. Fresnel reflectivity curves of light at 525 nm for 30 nm zinc and two overlayers (10 nm SiO_x + 30 nm PVA) are also shown to assess the extent of agreement between theoretical and the experimental data, that is fluorescence emission intensity should be the highest at the angles where the reflectivity is minimum due to coupling

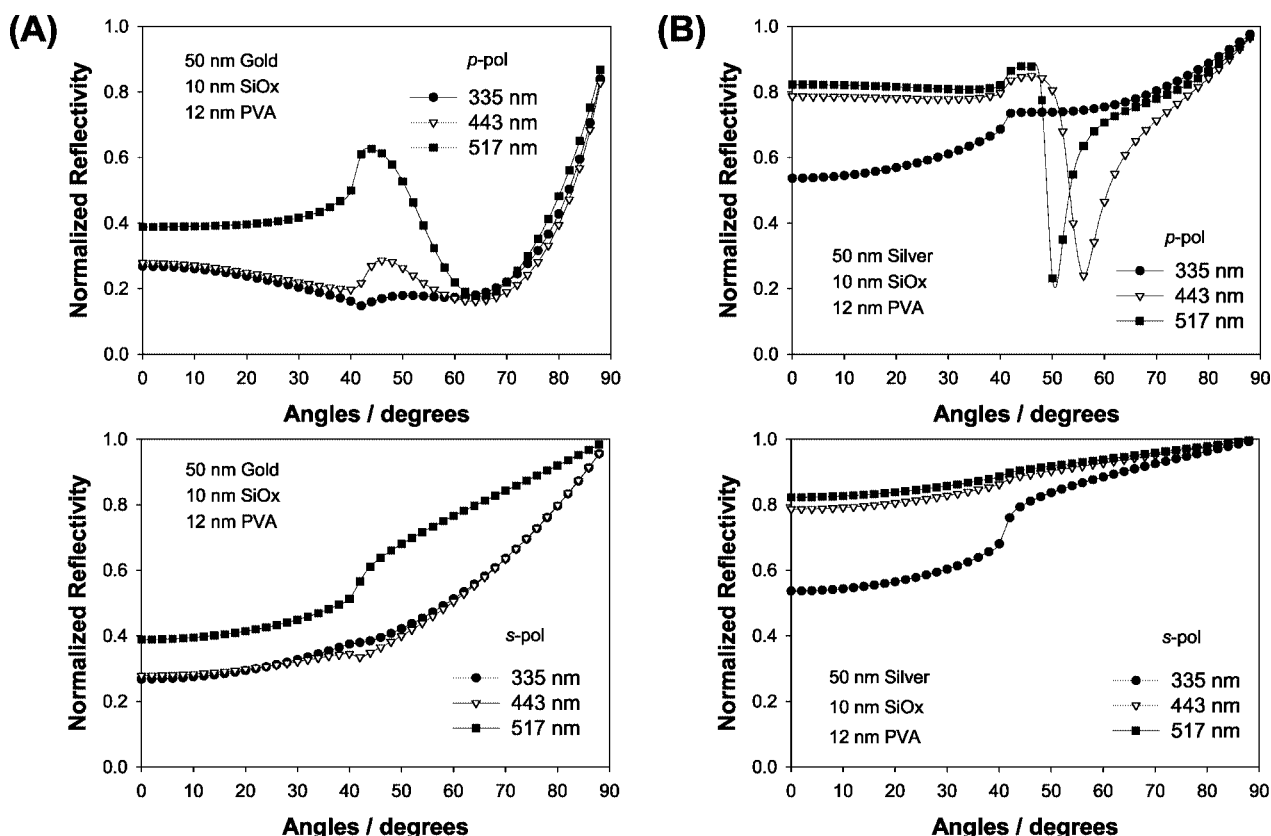


Figure 5. Wavelength-dependent reflectivity curves for gold and silver substrates for comparison with zinc and aluminum substrates. Five-phase Fresnel reflectivity curves of *p*- (top) and *s*- (bottom) polarized light at 335, 443, and 517 nm for 50 nm thick (A) gold substrates and (B) silver substrates with two subsequent overlayers of 10 nm SiO_x and 12 nm PVA.

of fluorescence emission to the surface plasmon modes. The angular distribution of SPCF emission from FITC mixed with 0.1% PVA overlaps with the Fresnel reflectivity curve for 30 nm zinc thin films and the SFCF intensity is the highest at 58°, giving us confidence in the data. Larger SFCF emission was observed as the initial concentration of PVA stock solution is further increased to 5 and 10%. The free-space emission and SPCF emission intensities were similar for all the samples used.

The Fresnel calculations for light at 525 nm in Figure 6B show that at 58°, while less than 10% of *p*-polarized light is expected to be reflected from the surface (or 90% is coupled to surface plasmons), 90% of *s*-polarized is reflected from the surface. The extent of *s*-polarized emission from FITC detected from the prism side was also determined experimentally. Figure 6C shows the ratio of *p*-polarized to *s*-polarized emission intensity of FITC (*P/S* ratio) increases as the initial concentration of PVA (all containing 50 μM FITC in the final mixture) spin coated onto zinc thin films is increased. This is due to the fact that more FITC molecules are present in the PVA films deposited from 5% and 10% PVA solutions, and thus the extent of *p*-coupling is larger.

It is important to discuss the significance of the discrimination of polarized light that can couple to surface plasmons. As mentioned before, *s*-polarized is a parallel dipole (with respect to the metal surface) and is canceled out by its own image on the metal surface while a perpendicular dipole (*p*-polarized) is coupled to the surface plasmon modes and is subsequently enhanced by the metal. In this regard, the SPCF emission measured from the back of the metal films is mostly *p*-polarized at an angle where the reflectivity of the metal is at minimum. In addition, as shown

in Figure 6C, the extent of coupling of *p*-polarized light increases with the increase in sample thickness.

It was previously reported that for a 10% PVA solution (prepared under different conditions as compared to the present study) *s*-polarized emission from fluorophores also coupled to surface plasmons at multiple observation angles.¹⁹ This was explained by the occurrence of waveguide modes in the samples with PVA thicknesses above 100 nm. It is important to note that, in the present study, *p*-polarized emission from FITC was detected from the prism side and *s*-polarized emission was negligible for samples prepared with 0.1% PVA. The coupling of *s*-polarized emission from FITC due to the waveguide modes was only observed for samples prepared with 10% PVA (Supporting Information, Figure S1).

Figure 7A shows the normalized *p*- and *s*-polarized fluorescence intensity from 100 μM POPOP in PMMA films deposited onto zinc thin films. The angular distribution of SPCF emission (*p*-polarized) from POPOP overlaps with the Fresnel reflectivity curve for 30 nm zinc thin films (calculated with 40 nm thick overlayers) and the largest SFCF intensity is measured at 68°. *s*-oriented POPOP molecules did not couple to surface plasmons and were not detected from the prism side. The SPCF measured at 425 nm from the prism side appears as a blue ring. Figure 7B shows the normalized *p*- and *s*-polarized fluorescence intensity from 3.5 mM 2-AP in PVA films deposited onto zinc thin films. The angular distribution of SPCF emission (*p*-polarized) from 2-AP overlaps with the Fresnel reflectivity curve for 30 nm zinc thin films (calculated with 20 nm thick overlayers) and the largest SFCF intensity is measured at 60°, i.e., the theoretical and experimental

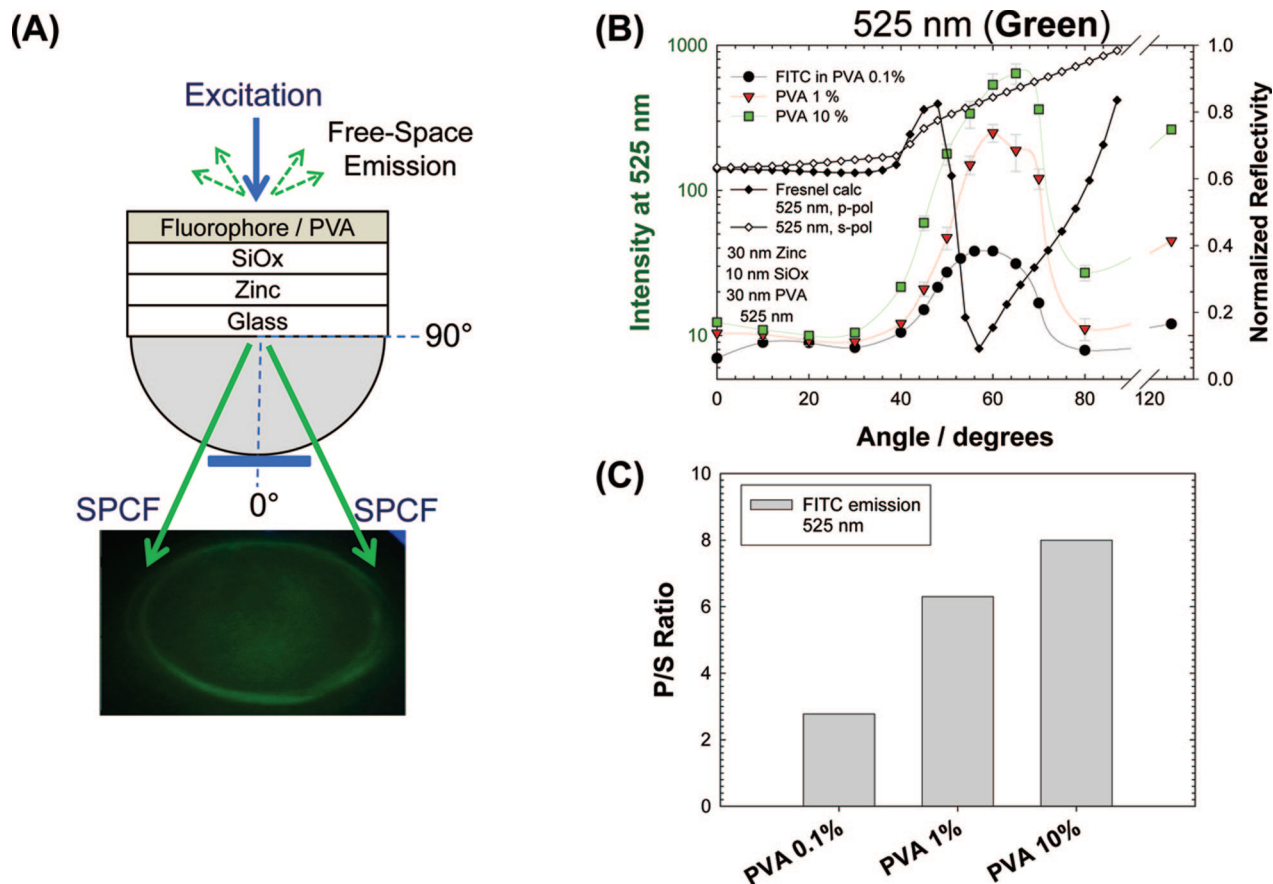


Figure 6. Experimental geometry and surface plasmon coupled fluorescence from FITC on zinc substrates. (A) Experimental geometry and detection scheme for zinc SPCF. The real-color photograph of a “green ring” resulting from surface plasmon-coupled fluorescence of FITC is also shown. (B) Five-phase Fresnel reflectivity curves of *p*- and *s*-polarized light at 525 nm for 30 nm thick zinc substrates with two subsequent overlayers of 10 nm SiO_x and a 30 nm PVA. Experimental normalized *p*- and *s*-polarized fluorescence intensity from 50 mM FITC in various concentrations of PVA film (emission is collected at 525 nm) on zinc substrates with 10 nm SiO_x overlayer. (C) Ratio of *p*-polarized to *s*-polarized emission intensity of FITC measured at 525 nm and at 58°. Data point at 125° represents the free-space emission.

data are in complete agreement. *s*-oriented fluorophores (POPOP) did not couple to surface plasmons and were not detected from the prism side. The *p*- and *s*-polarized emission intensities at lower angles (15–60° for POPOP and 5–40° for 2-AP) is significantly less than SPCF emission intensity and is thought to be due to the transmission of light through the zinc films (not surface plasmon coupled) as predicted by the Fresnel calculations.

The surface plasmons in metals generated by light are known to propagate as an evanescent wave along the metal/dielectric interface (*x*-direction).³⁶ The evanescent wave has an amplitude normal to the metal/dielectric interface that decays exponentially in the *z*-direction.³⁶ The penetration depth of this light into the dielectric is in the order of a few hundred nanometers, which also affords the opportunity for the selective coupling of excited states in close-proximity to the metal. Figure 8A shows the comparison for the calculated penetration depths for light at 517 nm above several metals. The penetration depth of 517 nm light into the water phase above zinc films is comparable to the penetration depths for other metal films used in SPCF. In this regard, fluorescence emission from fluorescent species in bioassays constructed above zinc thin films (Figure 8B) offers these important opportunities: (1) Any biospecies of interest can be potentially selectively detected using SPCF through a wide range of bioassays (immunoassays, DNA hybridization, ELISAs) constructed on zinc thin films. (2) The background coupling of

emission above zinc thin films (>20 nm, a typical thickness for a sandwich immunoassay) is significantly reduced, improving the sensitivity of fluorescence emission detected, (Figure 8B). Studies are underway in our laboratory in this regard. (3) Zinc is an inexpensive metal to fashion surfaces from, as compared to gold and silver. (4) The reflectivity and transmission curves can be predicted using multiphase Fresnel calculations as needed, where we have shown that experiments and theory closely match. (5) The Fresnel calculations show broad wavelength transmission, suggesting applicability to analytical sensing in the ultraviolet and visible spectral regions. (6) While silver and gold surfaces have been shown to be useful in the visible spectral regions, only aluminum substrates have to date been demonstrated in the ultraviolet spectral region. Zinc offers a useful low-cost alternative to aluminum but can also be used in the visible spectral region at the same time.

CONCLUSIONS

The use of zinc thin films in surface plasmon fluorescence spectroscopy (SPFS) is demonstrated. Theoretical Fresnel calculations predicted that light in the ultraviolet and visible spectral regions readily generate and couple to surface plasmon modes of zinc thin films. Additional Fresnel calculations performed to compare the efficacy of coupling of light to surface plasmons of aluminum, silver, and gold substrates showed that zinc thin

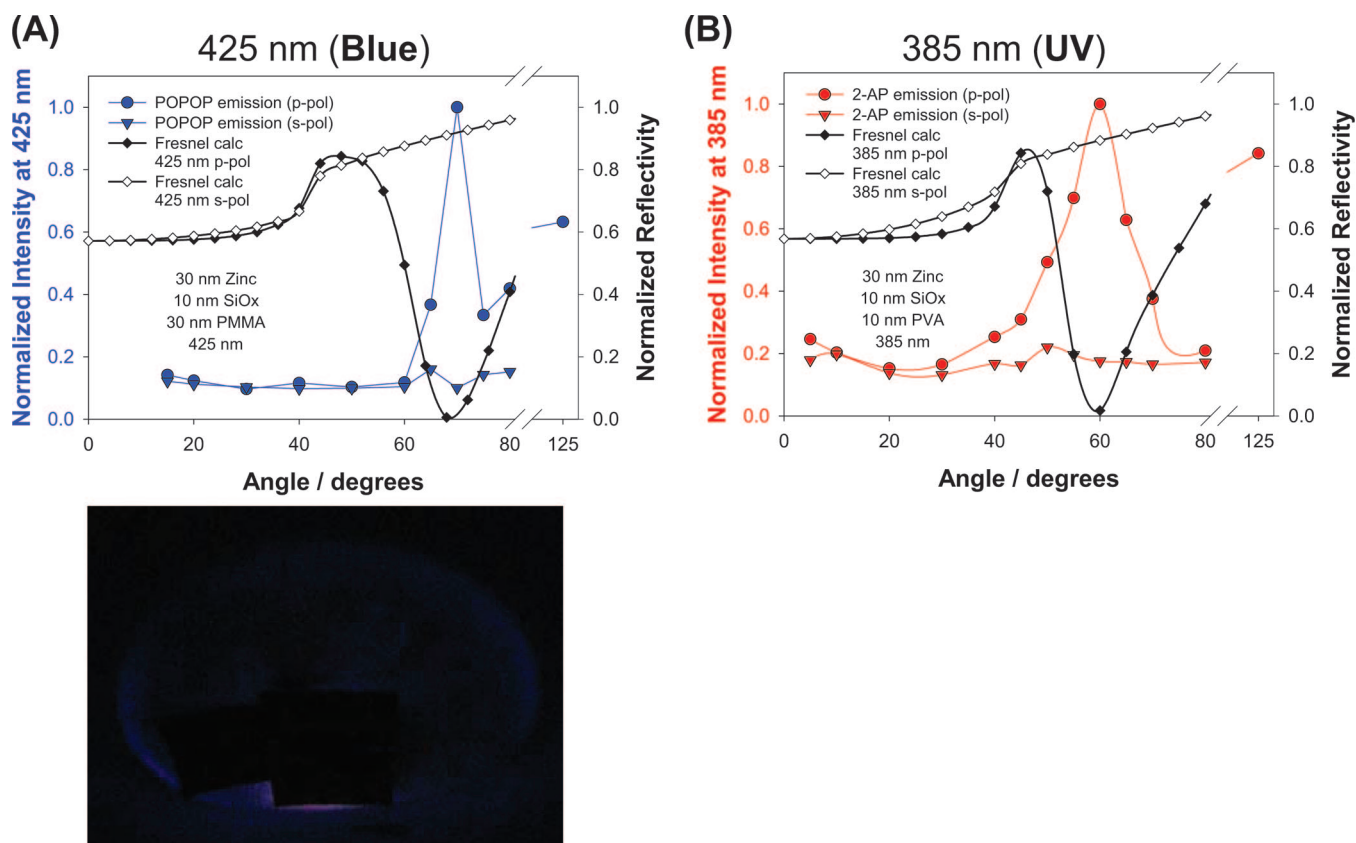


Figure 7. Surface plasmon coupled fluorescence from POPOP and 2-AP on zinc substrates. Five-phase Fresnel reflectivity curves of *p*- and *s*-polarized light at (A) 425 nm and (B) 385 nm for 30 nm thick zinc substrates with two subsequent overlayers of 10 nm SiO_x and a polymer. Experimental normalized *p*-polarized (●) fluorescence intensity from (A) 100 mM POPOP in 5% PMMA film (emission is collected at 425 nm) and (B) 3.5 mM 2-AP in 5% PVA film (emission is collected at 385 nm) on zinc substrates with a 10 nm SiO_x overlayer.

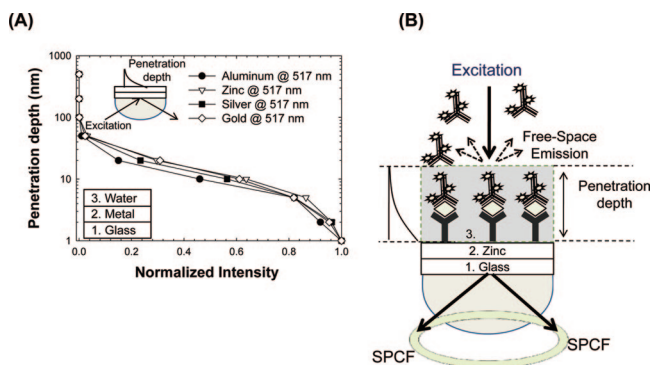


Figure 8. Potential bioassays using surface plasmon coupled fluorescence on zinc substrates. (A) A plot of calculated electric field intensity (E_z^2) for penetration depths above several metals using three-phase Fresnel calculations. (B) Opportunities for fluorescence-based detection of biospecies of interest based on SPCF using zinc thin films.

films can be used in SPFS over a 200 nm wavelength range where no single other metallic substrate can be used. The optimum thickness for zinc thin films was determined from Fresnel calculations to be ≈ 30 nm. The experimental demonstration of SPCF using zinc thin films in the ultraviolet and visible spectral regions was undertaken using three different fluorophores, FITC, POPOP, and 2-AP. The excitation of fluorophores spin coated onto zinc thin films using polymeric solutions in the reverse Kretschmann configuration resulted in the generation of polarized and directional emission (58, 68, and 60° for FITC,

POPOP, and 2-AP, respectively) from the prism side. The free-space emission and surface plasmon coupled fluorescence emission (*p*-polarized) intensities were measured to be similar for all the samples used. The coupling of *s*-oriented fluorophores to zinc thin films was negligible except for a FITC sample spin coated from a 10% PVA solution, which resulted in significant coupling due to the generation of waveguide modes on zinc thin films, consistent with similar observations made for other metals. These experimental results are shown to be in almost complete agreement with reflectivity curves that are theoretically predicted using multiphase Fresnel calculations. Zinc thin films offer the versatility of a single assay platform that can be used over a 200 nm wavelength range for applications in SPFS, potentially outperforming other single metallic substrates currently in use in analytical applications.

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SUPPORTING INFORMATION AVAILABLE

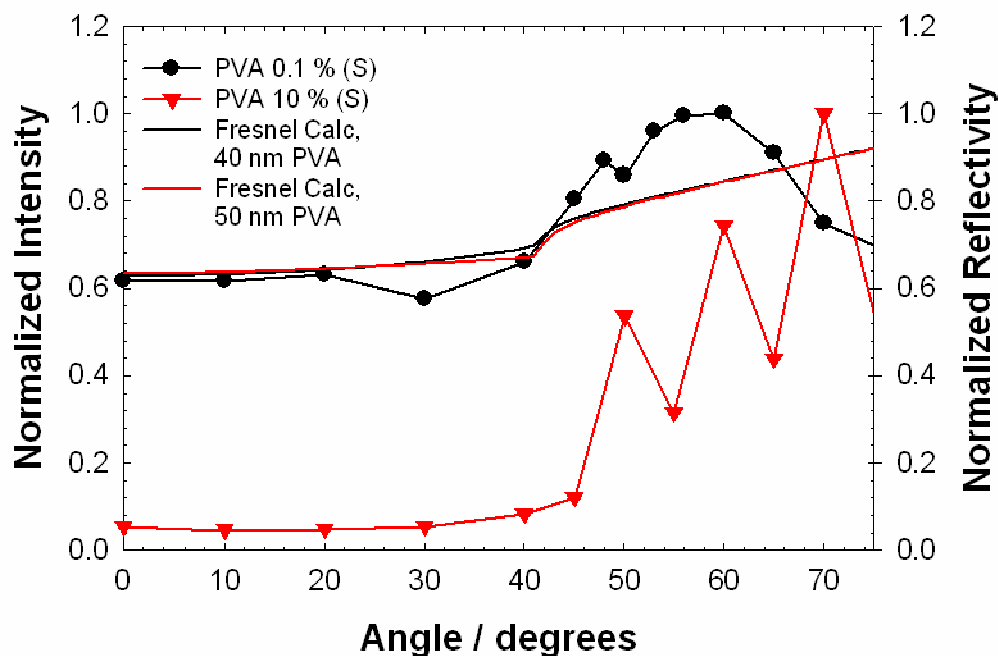
Additional information as noted in text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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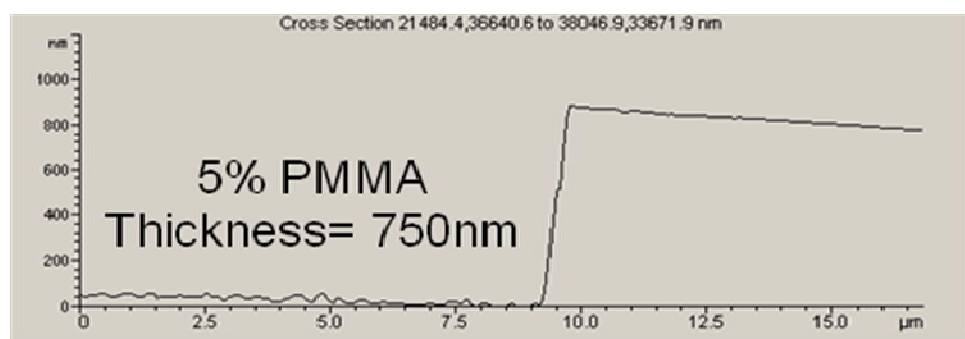
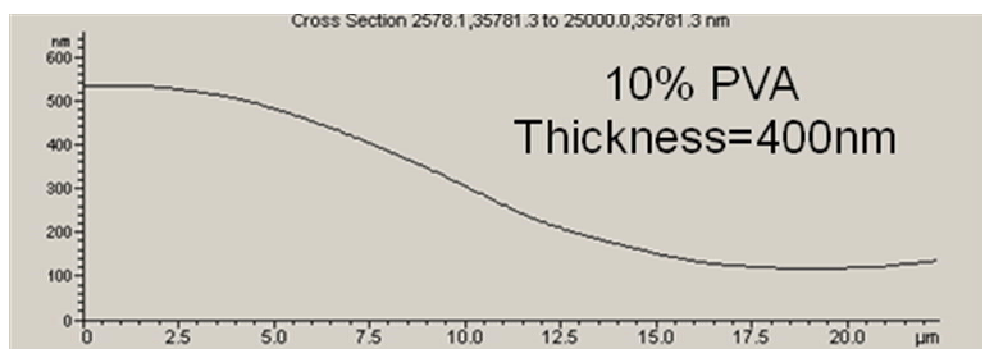
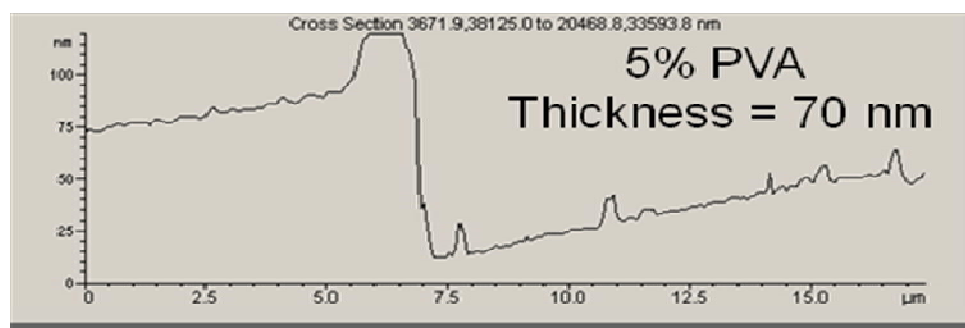
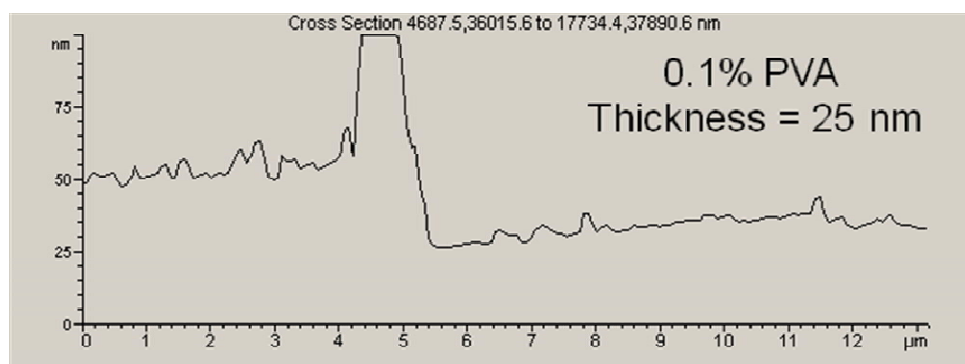
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Supporting Information for the Manuscript: "Surface Plasmon Coupled Fluorescence in the Ultra-Violet and Visible Spectral Regions using Zinc Thin Films" by Aslan et al.

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Supporting Information: Figure S1. Surface Plasmon Coupled Fluorescence from FITC on Zinc Substrates. Five-phase Fresnel reflectivity curves of *s*-polarized light at 525 nm for 30 nm thick zinc substrates with two subsequent overlayers of 10 nm SiO_x and a 40 nm PVA (solid black line) and 50 nm PVA (solid red line). Experimental normalized *s*-polarized fluorescence intensity at 525 nm from 50 mM FITC in various concentrations of a PVA film on zinc substrates with a 10 nm SiO_x overlayer. (0.1% PVA - solid circles; 10% PVA - inverted triangles).



Supporting Information: Figure S2. Thickness of polymer films measured using an Atomic Force Microscope.