

## Directional, Broad, and Fixed Angle Surface Plasmon Coupled Fluorescence from Iron Thin Films

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Fixed angle surface plasmon coupled fluorescence (SPCF) from iron thin films is demonstrated for the first time. The optimum thickness of the iron thin films was determined to be 15 nm using Fresnel calculations. The angles of minimum reflectivity for light at 496–814 nm were predicted to occur at a fixed and broad range of angles of 60–70°. Experimental corroboration of these predictions were undertaken by employing fluorescein isothiocyanate (emission peak at 517 nm), rhodamine B (580 nm), zinc phthalocyanine (710 nm), and IR 780 dye (814 nm). SPCF emission from all four fluorophores was directional, p-polarized, and observed at a fixed angle of ~65°.

Since the first introduction of surface plasmon fluorescence spectroscopy (SPFS),<sup>1</sup> a technique based on the near-field interactions of fluorophores and thin metal films, there has been significant publications related to its applications. In SPFS-based applications,<sup>2</sup> fluorophores are placed in close proximity to a thin metal film typically as part of a bioassay constructed on the surface of the metal film. The excitation of the fluorophores can be achieved directly from the sample side (referred to as reverse Kretschmann (RK) configuration) or through a prism (referred to as Kretschmann (KR) configuration). In the RK configuration, fluorophores are directly excited by the incident excitation source, and the resulting fluorescence emission induces and couples to surface plasmons. In the KR configuration, the excitation light entering through the prism generates the surface plasmons in the metal film and excites fluorophores within a certain distance (typically ~200 nm)<sup>1</sup> from the surface. The fluorescence emission can be detected from the sample side (free-space emission) and/or from the back of the metal film (surface plasmon coupled fluorescence, SPCF). While the free-space emission is isotropic, the predominantly p-polarized SPCF comes out of the prism at a specific angle; if a hemispherical prism is used, a “ring” of emission can be observed.<sup>3</sup>

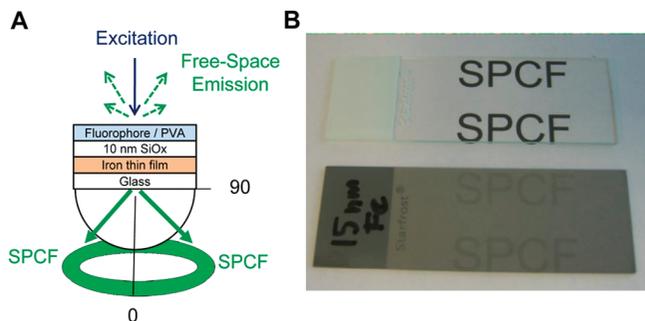
The SPCF angle coincides with the metal's angle of minimum reflectivity and varies with the type of metal used, subsequent overlayers present on the metal films, and the wavelength of the excitation source. The SPCF angle is narrow, especially for metals with a low  $\epsilon_i/\epsilon_r$  ratio ( $\epsilon_i$ , imaginary component of the dielectric function;  $\epsilon_r$ , real component of the dielectric function), such as gold,<sup>4</sup> silver,<sup>5</sup> and aluminum.<sup>6</sup> Subsequently, in applications based on these metal films, the detection optics must be adjusted for the varying observation angle, which can significantly increase the experimental error in the collection of data. In this regard, the data collection efficiency of the SPFS technique can be significantly improved by fixing the observa-

tion angle, similar to that of a traditional fluorescence spectrometer.

In this Letter, we subsequently present directional fluorescence from fluorophores that can be observed at a fixed angle from iron thin films. This is a significant optical advancement as compared to the first reports of SPCF, where the observation angle was shown to significantly change due to the presence of additional dielectric layers on the metal thin films. The data presented here suggest that metal thin films can be incorporated into existing fluorimeters (due to the fixed angle nature), which has not hitherto been reported. A potential drawback for using iron thin films in an existing fluorimeter is that the operating wavelength range would be limited to 496–814 nm as compared to 200–1100 nm for a typical fluorimeter.

Theoretical Fresnel calculations were employed to determine the optimum thickness of iron thin films to be 15 nm. The wavelength-dependent dielectric functions for iron thin films and most other metals are available in the CRC Handbook of Chemistry and Physics.<sup>7</sup> In addition, these functions can be determined experimentally for other metals.<sup>8</sup> Fresnel calculations also showed that light in the 496–814 nm wavelength range efficiently coupled to iron thin films and that the angle of minimum reflectivity was at a broad and fixed angle range of 60–70°. Iron thin films with a 10 nm thick SiO<sub>x</sub> overlayer were separately deposited onto silane-prep glass microscope slides by AccuCoat Inc., Rochester, NY, USA. The thickness of the deposited film was monitored by a quartz crystal microbalance. To corroborate the theoretical predictions, SPCF from four fluorophores emitting in the 486–814 nm wavelength range were measured using a hemispherical prism and a commercially available spectrofluorometer (Ocean Optics, Inc., Florida, USA). The method for the deposition of fluorophores onto metallic thin films was published elsewhere.<sup>9</sup> Fluorophores were deposited onto iron thin films by spin-coating a solution of polymers containing the fluorophores. The fluorophores were excited using the RK configuration, Figure 1A. The fluorophores fluorescein isothiocyanate (FITC, emission peak at 517 nm), rhodamine B

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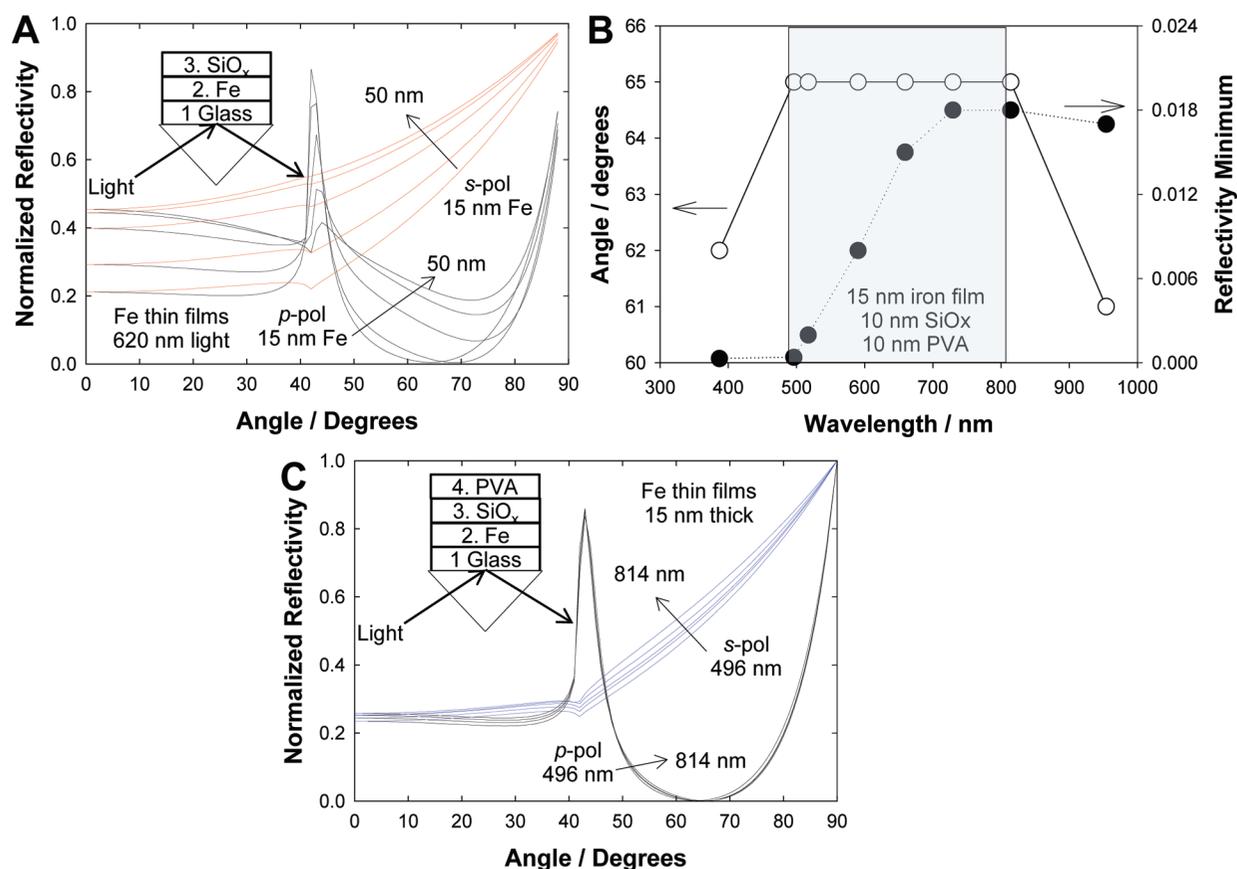


**Figure 1.** (A) Schematic representation of the experimental setup for surface plasmon coupled fluorescence (SPCF) measurements carried out with a hemispherical prism which was used to collect the “ring” of emission. (B) Photographs of 15 nm iron thin films as compared to a blank glass slide. The text below the slides demonstrates the semi-transparent nature of the films.

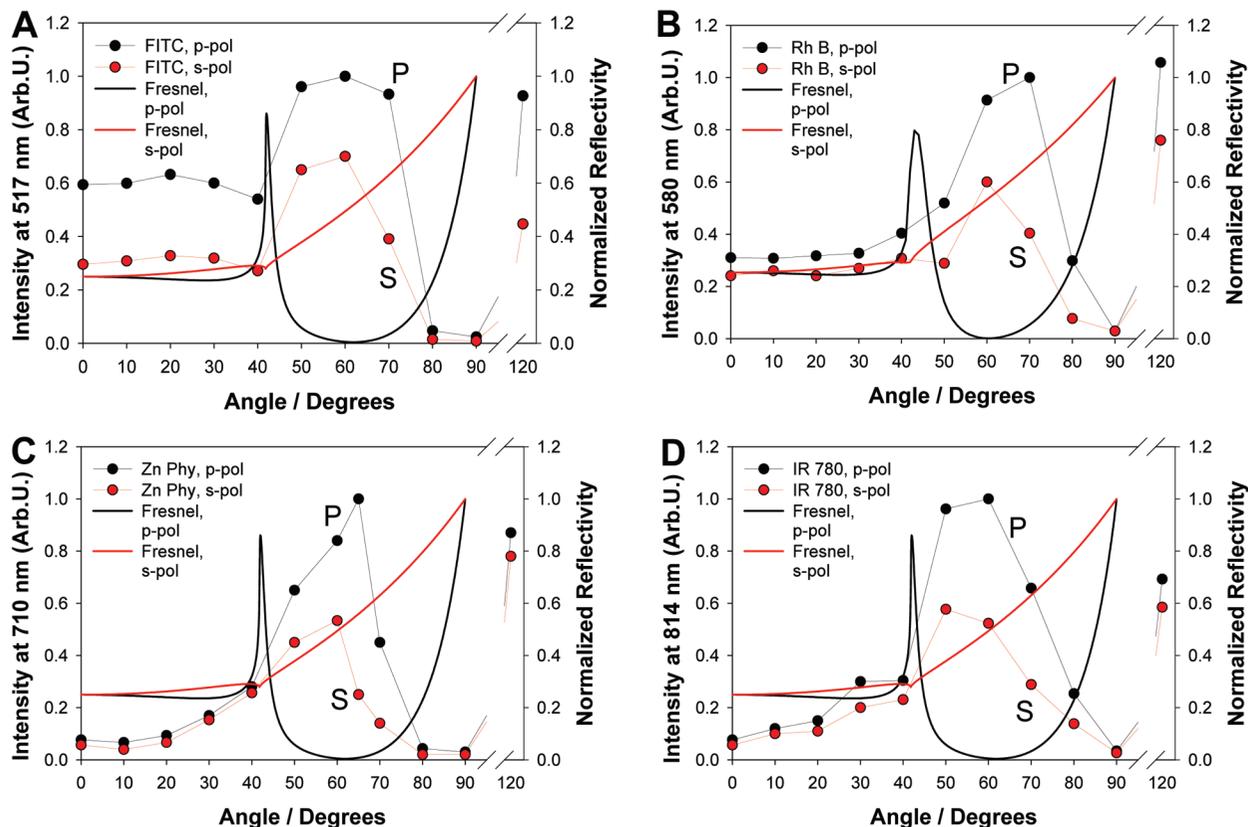
(Rh B, 580 nm), zinc phthalocyanine (Zn Ph, 710 nm), and IR 780 dye (814 nm) were mixed in a solution of polyvinyl alcohol (PVA) and spin-coated onto the iron thin films, which created a  $\sim 10$  nm thick layer of PVA.<sup>9</sup> SPCF from fluorophores deposited onto iron thin films was found to be highly directional at a fixed angle of  $\sim 65^\circ$ , as predicted by Fresnel calculations. Free-space emission (measured at  $120^\circ$ ) was isotropic and had similar intensity values as SPCF at  $65^\circ$ . Figure 1B shows the photographs of 15 nm iron thin films as compared to a blank glass slide. The text below the slides demonstrates the semi-transparent nature of the films.

Figure 2A shows the three-phase Fresnel reflectivity curves for p- and s-polarized light (620 nm) incident upon various thicknesses of iron thin films with a 10 nm  $\text{SiO}_x$  overlayer. Fresnel calculations show that a reflectivity curve has a minimum at angles between  $60$  and  $70^\circ$  for  $15$ – $20$  nm thick iron thin films, and thus, one can choose either thickness for SPCF. It is important to note that additional overlayers typically result in further shift in the angle of minimum reflectivity toward  $90^\circ$ ,<sup>5</sup> which presents physical challenges in data collection.<sup>9</sup> Thus, SPCF measurements are recommended to be performed at observation angles  $< 70^\circ$ , when possible. Subsequently, in this study, 15 nm thick iron thin films were chosen. The reflectivity curves for thicker iron thin films ( $>40$  nm) do not show a reflectivity minimum and are deemed not useful for SPCF. The  $\epsilon_i/\epsilon_r$  ratio for iron is much larger than that for gold and silver; subsequently, the minimum in the reflectivity curves is broader compared to that of gold and silver.

To determine the range of fluorophores that will most efficiently couple to iron thin films with additional overlayers, four-phase Fresnel reflectivity curves were calculated for a range of wavelengths (387–954 nm). Figure 2B shows the plot of reflectivity minimum angles and the reflectivity minimum (for p-polarized light) versus the wavelength of incident light for 15 nm iron thin films with a 10 nm  $\text{SiO}_x$  and a 10 nm overlayer. Figure 2B reveals that the observation angle for SPCF emission over the 496–814 nm wavelength range should occur at a fixed angle of  $65^\circ$ . It is also important to note that the variation in the values for the reflectivity minimum for p-polarized light (y-axis right) over the range of wavelengths is very small. The



**Figure 2.** (A) Four-phase Fresnel reflectivity curves for p- and s-polarized light at 620 nm for iron thin film thicknesses at 15, 20, 30, 40, and 50 nm with a 10 nm  $\text{SiO}_x$  overlayer. (B) A plot of reflectivity minimum angles and reflectivity minimum (for p-polarized light) versus the wavelength of incident light for 15 nm iron thin films with a 10 nm  $\text{SiO}_x$  and a 10 nm overlayer. Minimum reflectivity occurs at a fixed angle of  $65^\circ$  for light at 496–814 nm. (C) Four-phase Fresnel reflectivity curves for p- and s-polarized light at 496, 517, 620, 710, and 814 nm for 15 nm iron thin films with a 10 nm  $\text{SiO}_x$  and 10 nm PVA overlayers.



**Figure 3.** Experimental p- and s-polarized emission collected (A) at 517 nm from fluorescein isothiocyanate (FITC), (B) at 580 nm from rhodamine B (Rh B), (C) at 710 nm from zinc phthalocyanine (Zn Phy), and (D) at 814 nm from IR 780 dye. Five-phase Fresnel reflectivity curves showing p- and s-polarized light (corresponding to the emission wavelength of each fluorophore) for 15 nm iron thin films with 10 nm  $\text{SiO}_x$  and 10 nm PVA overlayers are also plotted to demonstrate the close agreement between the experimental data and the theoretical calculations.

calculated reflectivity curves for p- and s-polarized light at the wavelength range of 496–814 nm are shown Figure 2C. Figure 2C shows that the light which couples to surface plasmons at  $65^\circ$  is predominantly p-polarized. The extent of coupling of s-polarized light at  $65^\circ$  is also predicted to decrease as the wavelength of light is increased.

Figure 3 shows the experimental SPCF emission from selected fluorophores and the calculated Fresnel reflectivity curves for light at the wavelength corresponding to the wavelength of the emission peak of a fluorophore. It is important to note that Figure 3 is plotted to demonstrate the extent of agreement between the experimental data and theoretical predictions. Figure 3A shows normalized p- and s-polarized fluorescence intensity from 50  $\mu\text{M}$  FITC in PVA films deposited onto iron thin films. The SPCF emission from FITC-coated iron thin films is measured at angles of  $0$ – $90^\circ$ , and the free-space emission is measured at  $120^\circ$ . Fresnel reflectivity curves of light at 517 nm for 15 nm iron thin films and an overlayer of 10 nm  $\text{SiO}_x$  and 10 nm PVA are also shown. Figure 3A shows that fluorescence emission intensity at 517 nm is the highest at the angles where the reflectivity is minimum due to coupling of fluorescence emission to surface plasmons in iron thin films, suggesting the close agreement between theoretical and the experimental data. The free-space emission intensity was similar to that of the SPCF intensity measured at  $65^\circ$ .

Figure 3B–D shows p- and s-polarized fluorescence intensity from 50  $\mu\text{M}$  Rh B (at 580 nm), 50  $\mu\text{M}$  Zn Phy (at 710 nm), and 50  $\mu\text{M}$  IR 780 dye (at 814 nm) in PVA films deposited onto iron thin films, respectively. The maximum SPCF emission intensity from all three fluorophores also occurred at  $65^\circ$ . Interestingly, the extent of coupling of s-polarized emission from Rh B, Zn Phy, and IR 780 dye was less as compared to that for

FITC. As indicated before, theoretical Fresnel calculations predicted the decrease in the extent of coupling of s-polarized light to the iron thin films (see Figure 2C).

It is interesting to discuss the reasons for the discrimination of polarized light that induces/couples to surface plasmons in metals. Near metal surfaces, a parallel dipole (s-polarized light) is canceled out by its own image on the metal surface, while a perpendicular dipole (p-polarized light) induces surface plasmon modes.<sup>10</sup> Subsequently, p-polarized emission from fluorophores near metals can induce/couple to surface plasmons and is preferentially emitted from the back of the metal film at an angle where the reflectivity of the metal is at a minimum. Since Fresnel calculations predict the interactions of light with metal thin films, they are an important tool for the design of metal surfaces for SPCF applications.

In conclusion, fixed angle surface plasmon coupled fluorescence from iron thin films is presented. Fresnel calculations predicted the optimum thickness of iron thin films to be 15 nm for SPCF applications. The range of wavelengths of light that can efficiently induce and couple to surface plasmons in iron thin films was predicted to be 387–954 nm. In addition, the angle of minimum reflectivity for light at 496–814 nm was predicted to occur at a fixed and broad range of angles of  $60$ – $70^\circ$ , centering at around  $65^\circ$ . Experimental data for SPCF from fluorophores with emission peaks within 496–814 nm showed a very close agreement with predictions of the Fresnel calculations. Since the SPCF from iron thin films can be observed at a fixed angle for the wide wavelength range, no adjustment in the angle of detection optics is necessary. This is especially attractive for the incorporation of metal thin films into existing fluorescence technology and for the development of a generic fluorometer that is based on SPCF.

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#### References and Notes

- (1) Liebermann, T.; Knoll, W. *Colloids Surf., A* **2000**, *171*, 115–130.
- (2) Liebermann, T.; Knoll, W.; Sluka, P.; Herrmann, R. *Colloids Surf., A* **2000**, *169*, 337–350.

- (3) Lakowicz, J. R. *Anal. Biochem.* **2004**, *324*, 153–169.
- (4) Gryczynski, I.; Malicka, J.; Gryczynski, Z.; Lakowicz, J. R. *J. Phys. Chem. B* **2004**, *108*, 12568–12574.
- (5) Matveeva, E.; Malicka, J.; Gryczynski, I.; Gryczynski, Z.; Lakowicz, J. R. *Biochem. Biophys. Res. Commun.* **2004**, *313*, 721–726.
- (6) Gryczynski, I.; Malicka, J.; Gryczynski, Z.; Nowaczyk, K.; Lakowicz, J. R. *Anal. Chem.* **2004**, *76*, 4076–4081.
- (7) Lide, D. R. *CRC Handbook of Chemistry and Physics*; CRC Press: Boca Raton, FL, 2004–2005.
- (8) Jiao, X.; Goeckeritz, J.; Blair, S.; Oldham, M. *Plasmonics* **2009**, *4*, 37–50.
- (9) Aslan, K.; Previte, M. J.; Zhang, Y.; Geddes, C. D. *Anal. Chem.* **2008**, *80*, 7304–7312.
- (10) Barnes, W. L. *J. Mod. Opt.* **1998**, *45*, 661–699.

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