Surface plasmon coupled chemiluminescence from zinc substrates: Directional chemiluminescence

Kadir Aslan and Chris D. Geddes[a)]
The Institute of Fluorescence, Medical Biotechnology Center, University of Maryland Biotechnology Institute, 701 East Pratt St, Baltimore, Maryland 21202, USA

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In this letter, the observation of surface plasmon coupled directional chemiluminescence (SPCC) from zinc thin films is reported. Fresnel calculations predict that light in the ultraviolet and visible spectral range can induce surface plasmon modes in 30 nm zinc thin films. Free-space emission from blue and green chemiluminescent solutions on zinc thin films was isotropic, and the SPCC emission was highly directional and *p*-polarized in accordance with the Fresnel calculations. In addition, the decay rates of the free-space and SPCC emission were similar, which suggests that zinc thin films have no catalytic effect on chemiluminescence. © *2009 American Institute of Physics*. [DOI: 10.1063/1.3082175]

Chemiluminescence is a powerful analytical tool for the quantitative detection of a variety of biological materials such as cells, micro-organisms, proteins, DNA, RNA, and small molecules, and is currently employed in more than 20 % of clinical laboratories in the USA.[1] In chemiluminescence-based detection, chemiluminescence emission is generated by enzyme-catalyzed chemical reactions where there is no need for an excitation source and optical filters as compared to fluorescence.[2] However, chemiluminescence-based detection is limited by the quantum efficiency of the chemiluminescence reaction or probe, which invariably results in poor signal-to-noise ratios at low analyte concentrations in addition to long detection times. Consequently, there is a continued search for methods to increase the chemiluminescence yield and to accelerate the chemical reactions that result in chemiluminescence emission. In this regard, a technique based on the interactions of light with metal thin films, called surface plasmon coupled chemiluminescence (SPCC) was recently reported by our laboratory[3,4] In SPCC, chemically induced electronic excited states induce surface plasmon modes in thin metal films and the coupled emission is subsequently emitted from the back of the metal thin films with a preferential polarization at a specific angle, i.e., is both highly directional and polarized.[3] In close proximity to the metal surface (<200 nm), SPCC emission is reportedly larger than that of free-space emission due to efficient coupling (induced surface dipole) of emission to surface plasmons.[3] Since most biological assays that are constructed on planar surfaces require the placement of enzymes close to surfaces through biological recognition events, the resulting chemiluminescence emission typically occurs within a few hundred nanometers away from the surface. In this regard, metal thin films are proven to be alternative assay platforms for a variety of surface plasmon coupled luminescence (SPCL)-based bioassays.[5,6] Typically, gold and silver thin films are the choice of surfaces in SPCL-based applications due their versatility, i.e., these films are inert and amenable to chemical modification without losing both their physical and electronic properties. Despite their versatility, these thin films are moderately expensive to produce. To alleviate this problem, other metals, which also support the generation of surface plasmons such as aluminum,[7] copper,[8] and zinc,[9] were proposed as alternative assay platforms to gold and silver thin films in surface plasmon coupled fluorescence applications.

In this letter, the use of zinc thin films for SPCC is demonstrated. The optimum thickness of the zinc thin films for efficient coupling of light in the ultraviolet and visible spectral range was determined to be 30 nm using theoretical Fresnel calculations, as previously reported.[9] An overlayer of SiOx was also employed to protect the zinc thin films from oxidation and to separate the chemiluminescence dye solutions from the surface. Free-space and SPCC emission from blue and green emitting chemiluminescent dyes placed on zinc thin films were measured using a hemispherical prism and a commercially available spectrofluorometer (Ocean Optics, Inc., Florida, USA). SPCC emission was mostly *p*-polarized and was observed at specific angles as predicted by theoretical Fresnel calculations as compared to traditional isotropic free-space emission. In addition, similar decay rates for both the free-space and directional SPCC emission were observed, which suggests zinc thin films do not have a catalytic effect on the chemical reactions that yield chemiluminescence emission.

The blue and green chemiluminescent dyes (from Omnioglow, West Springfield, MA) were part of a chemilumi-

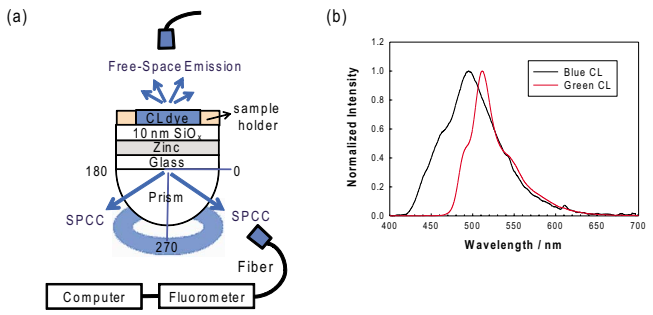

FIG. 1. (Color online) (a) Schematic of the experimental setup for SPCC measurements carried out with a hemispherical prism from zinc thin films (b) emission spectrum of chemiluminescent dyes used in this study.

a)Author to whom correspondence should be addressed. Electronic mail: geddes@umbi.umd.edu.

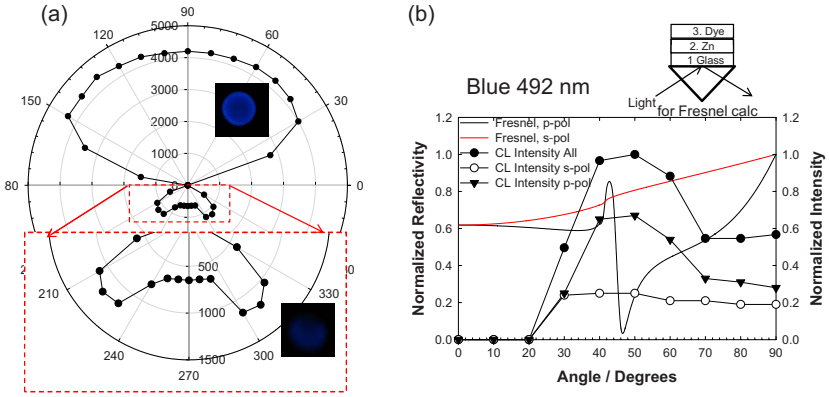

FIG. 2. (Color online) (a) Polar plot of free-space blue chemiluminescence emission (0°–180°) and SPCC (180°–360°). Real-color photographs of free-space emission and SPCC are also shown. (b) Three-phase Fresnel reflectivity curves showing *p*- and *s*-polarized light for 30 nm zinc thin films at 492 nm, corresponding to the emission peak of the blue chemiluminescence dye. Experimental *p*- and *s*-polarized emission were collected at 492 nm from a 100 microliters dye solution placed on the zinc thin films.

nescent kit that contains the necessary chemicals to produce chemiluminescence emission, which were encapsulated inside a plastic tube. The chemicals [hydrogen peroxide and diphenyl oxalate (blue) or Bis(phenylethynyl)oxalate (green)] were also compartmentalized in separate glass tubes inside the plastic tube. A very intense chemiluminescence emission lasting at least 2 h was observed after the glass tubes were broken and the chemicals were completely mixed.

Figure 1(a) shows the depiction of the experimental geometry for SPCC measurements. In this regard, zinc thin films with a SiOx overlayer were placed on top of a hemispherical prism. A sample holder (diameter × depth, 9×2 mm^2, volume capacity: 100 μl) were attached to the zinc thin films and a 100 μl of the freshly mixed chemiluminescent solution was placed inside the sample holder. A fiber optic that is connected to a fluorometer is used to record the chemiluminescence emission spectrum and emission intensity at observation angles of 0°–360°. Figure 1(b) shows the chemiluminescence emission spectrum of the two chemiluminescent dyes used in this study. The emission peaks occur at 492 and 509 nm for the blue and the green chemiluminescent dyes, respectively. These wavelengths are similar to those observed from commercially available chemiluminescence assays, which employ acridan-based chemical reactions. In addition, one can also find chemiluminescence kits that yield red emission (Invitrogen Corp.)

Figure 2(a) shows the polar plot of blue chemiluminescence emission intensity at 492 nm measured from the sample side (free-space emission, 0°–180°) and from the back of the hemispherical prism (directional SPCC, 180°–360°). An isotropic free-space emission was observed from the sample side. It is important to note that due to the experimental geometry (the sample holder has a depth of 2 mm above the hemispherical prism) free-space emission intensities at observation angles 0°–10° and 170°–180° were partially blocked by the sample holder and thus were significantly less than the other free-space emission intensities. On the other hand the sample holder did not affect the measurements from the back of the hemispherical prism. Figure 2(a) inset shows the directional SPCC emission at observation angles 210°–230° and 300°–320°. It is also important to note that SPCC is emitted as a "ring," as depicted in Fig. 1(a), due to surface dipole symmetry conditions, in contrast with traditional chemiluminescence; the emission is both highly directional and polarized. Thus directional SPCC emission is observed at 210°–230° and 300°–320°. Real-color photographs are shown to visually demonstrate free-space emission and SPCC, which also show that free-space emission is brighter. It is also important to note the observation of larger free-space emission intensities, which is thought to be mainly due the thickness of the chemiluminescent solution placed in the sample holder, where a depth ≈2 mm depth sample holder was used. Since the chemiluminescence emission from a thinner sample decays faster than the time it takes for measurements to be taken at all angles and polarizations, the sample thickness was kept at 2 mm to collect data of those that are not affected by the decrease in chemiluminescence emission. In a real-world application based on SPCC one would measure the emission from a sample typically <200 nm in thickness and at a fixed angle in a short period of time, rather than at all angles as done in this study. Subsequently, the authors note that one would observe larger SPCC intensities from chemiluminescent samples of <200 nm in thickness, and thus the signal-to-noise ratio will be larger.

The qualitative comparison of the experimental angular-dependent SPCC data with theoretical Fresnel calculations

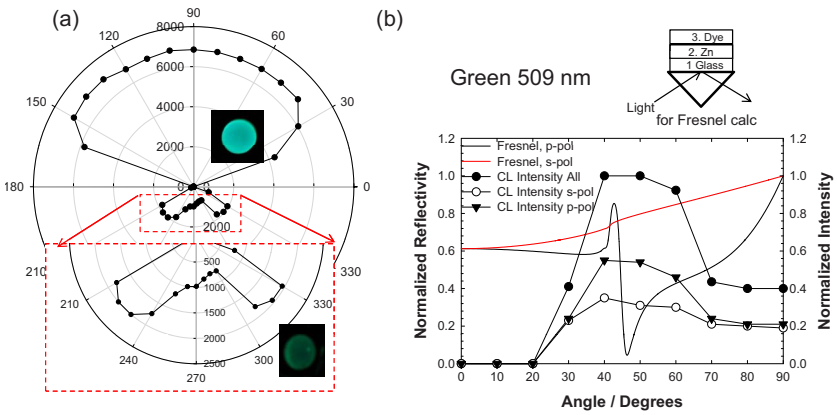

FIG. 3. (Color online) (a) Polar plot of free-space green chemiluminescence emission (0°–180°) and SPCC (180°–360°). Real-color photographs of free-space emission and SPCC are also shown. (b) Three-phase Fresnel reflectivity curves showing *p*- and *s*-polarized light for 30 nm zinc thin films at 509 nm, corresponding to the emission peak of the green chemiluminescence dye. Experimental *p*- and *s*-polarized emission were collected at 509 nm from a 100 microliters dye solution placed on the zinc thin films.

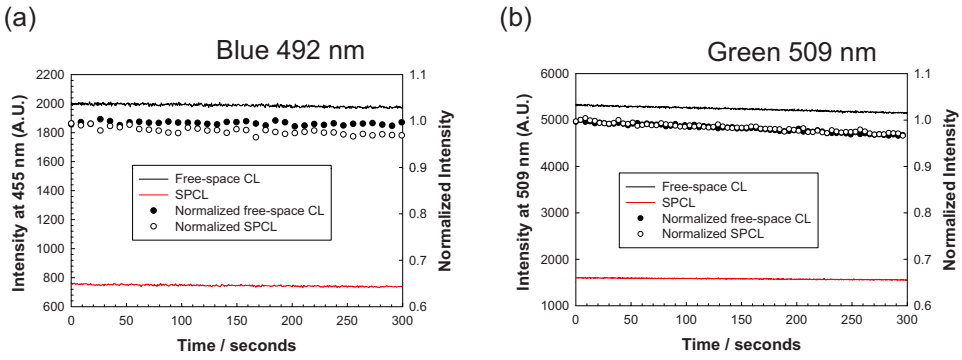

FIG. 4. (Color online) Time course decays of free-space and SPCC emissions of (a) blue dye, intensity measured at 492 nm (b) green dye, and intensity measured at 509 nm. Normalized emission intensities for free-space and SPCC are also shown to compare the decay rates.

were also undertaken and shown in Fig. 2(b). It is important to note that Fresnel calculations are carried out for an evanescent wave [Fig. 2(b) inset] created by light entering the prism at a critical angle (42.7°). Surface plasmons can efficiently be generated by light less than a few hundred nanometers above the metal surface at angles where the reflectivity is minimum. Light can induce surface plasmons at these angles and is emitted from the back of the prism. Since there is no external excitation source in chemiluminescence, the results of Fresnel calculations can be used to predict the wavelength and angular dependent generation of surface plasmons in metals to which chemiluminescence emission can couple. It is important to note that a direct comparison of the angles for the occurrence of reflectivity minimum (theoretical) and emission intensity maximum (experimental) can be made. Figure 2(b) shows that the light can induce surface plasmons in zinc thin films and the coupled light can be emitted from the back of the film with a maximum intensity occurring at ≈52° (Fresnel calculations). Figure 2(b) also shows the measured unpolarized, *p*-polarized, and *s*-polarized chemiluminescence emission intensity data [corresponding to data at 270°–360° in Fig. 2(a)] superimposed on the Fresnel calculations. The component of *p*-polarized light was significantly larger than that of the *s*-polarized light. In addition, while *p*-polarized light was preferentially coupled and emitted from to zinc thin films, *s*-polarized light appears as a constant background signal.

Figure 3 summarizes the results for a green chemiluminescent dye. The interpretation of the data presented in Fig. 3 is similar to that for the blue chemiluminescent dye. Free-space emission for the green chemiluminescent dye is larger than the SPCC emission for the reasons described for the blue chemiluminescent dye. Fresnel calculations predict the angle of reflectivity minimum for 509 nm light at 50° with a non-100% value. In addition, the component of measured *s*-polarized light is larger than that for 492 nm light. These results demonstrate that chemiluminescence excited states couple to surface plasmons in zinc thin films and are preferentially *p*-polarized and emitted at specific observation angles, two major criteria for SPCC, as predicted by theoretical Fresnel calculations.

In our previous publications on SPCC,[3,4] the question of whether there is a catalytic effect of metals on the chemical reactions that generate chemiluminescence has arisen. To investigate whether zinc thin films indeed have a catalytic effect on the chemiluminescence emission, the decay of free-space emission [at 90°, Fig. 1(b)] and SPCC emission [310°, Fig. 1(b)] intensity over time was measured, c.f., Fig. 4. Subsequently, free-space and SPCC emission intensity values were normalized to compare the rate of decay of each intensity [Figs. 4(a) and 4(b)]. The decay rate for both free-space and SPCC emissions were very similar indeed, which suggests zinc thin films do not have a catalytic effect on the chemical reactions that yield chemiluminescence emission. It is important to note that an overlayer of SiOx was employed in these sample geometries, which prevented a direct contact between the chemiluminescent solutions and the zinc thin films, so this finding is not too surprising.

In conclusion, the demonstration of surface plasmon coupled *highly directional* chemiluminescence from 30 nm thick zinc thin films is presented. Blue and green SPCC emission from the chemiluminescent solutions were preferentially *p*-polarized and were emitted from the back of the prism at 210°–230° and 300°–320°. The decay rates of free-space and SPCC chemiluminescence emissions were similar, strongly suggesting that zinc thin films with a 10 nm SiOx overlayer do not have a catalytic effect on the chemical reactions that generate the chemiluminescence emission. Given the widespread use of chemiluminescence in diagnostics today and the fact that most chemiluminescence in these optical trains is not efficiently collected due to the isotropic emission, then our work on highly directional and polarized chemiluminescence will be of significance. Further plasmon-enhanced chemiluminescence work is underway in our laboratory and will be reported in due course.

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