First Observation of Surface Plasmon-Coupled Emission Due to LED Excitation

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Detection limitations for fluorescence methods are normally determined by the phenomenon itself rather than the sensitivity of the instrumentation. These limitations make it necessary to have high sensitivity, high cost equipment causing fluorescence methods to remain lab-oriented. Alleviation of the limitations can be achieved through the phenomenon of surface plasmon-coupled emission (SPCE), which displays enhanced, directional, polarized fluorescence. Here we present the excitation of SPCE from Rhodamine B with a light-emitting diode (LED). Incorporating the gains in sensitivity due to SPCE with LED excitation, it could be possible to design low-cost, high-sensitivity sensors that would allow measurements to be performed in the field.

INTRODUCTION

Fluorescence spectroscopy has become one of the dominant tools for assays in the biological sciences, as well as in biochemical and biomedical research. The various fluorescence observables, including lifetimes, spectra, anisotropies, and quantum yields, have all been utilized to increase the sensitivity of assays. However, the sensitivity is typically limited by the background fluorescence of the sample. The detection limits are also hindered by the use of the free-space emission of fluorophores, which is isotropic in all directions resulting in approximately 1% of the total emission being collected [1]. These limitations to fluorescence spectroscopy have caused it to remain mainly lab-oriented due to the equipment necessary to perform measurements.

Recently, in several experimental [2–9] and theoretical [10,11] studies, a phenomenon termed surface plasmon-coupled emission (SPCE) was described which could significantly lower the detection limits of current fluorescence methods. SPCE is similar to the phenomenon of surface plasmon resonance (SPR) [12–14]. In SPR, resonant oscillations of the free electrons on a thin metallic surface are created when light is incident through a prism of moderate refractive index at the SPR angle. The SPR angle is the angle at which the wavevector of the incident light equals the wavevector of the surface plasmons, which is seen through a decrease in the reflectance as the light is being absorbed.

SPCE can be thought of as the reverse process of SPR where emission is studied as opposed to absorbance. The phenomenon occurs for fluorophores within 200 nm of a thin metal film making this a near-field phenomenon [4,10]. The excited fluorophores couple with the surface plasmons which in turn radiate into the prism at the resonance angle (where wavevector matching occurs) for the emission wavelength. The SPR angle is highly dependent on sample thickness allowing for SPR studies of bioaffinity surface reactions [15–18] and for SPCE bioassays [19–23].

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The studies of SPCE showed that the excitation did not have to be at the resonance angle, suggesting any excited fluorophore near a thin metal film would display SPCE. This observation led to studies of electrochemically excited SPCE [8]. To date, all other studies of SPCE have been performed using laser excitation. Here we report the first observation of SPCE excited by a light-emitting diode (LED). Incorporation of the high-sensitivity gains of SPCE and the use of LED excitation could lead to low-cost, handheld instrumentation revolutionizing fluorescence spectroscopy.

MATERIALS AND METHODS

Sample Preparation

A 50-nm thick layer of silver, followed by a 5-nm thick layer of SiO_2, was vapor deposited on a glass microscope slide (plain, Corning) by EMF Corp. (Ithaca, NY). The SiO_2 layer protects the silver layer from oxidation and serves as a spacer. A 4 mM solution of Rhodamine B in 1% poly(vinyl alcohol) (PVA) was deposited on the silica surface and then spin-coated at 3000 rpm to create a thin layer. It is estimated from previous SPCE studies that a 1% PVA solution spin-coated at 3000 rpm creates approximately a 40 nm layer [7]. Using the equations from SPR theory, the reflectance profile for the radiated light from SPCE can be calculated to determine the angle at which the radiation will occur for the system. These equations can be found in the literature [24,25]. Reflectance calculations can also be performed using TFCalc 3.5 software (Software Spectra, Inc., Portland, OR).

Fluorescence Measurements

The slide spin-coated with the sample was attached to a hemi-cylindrical prism using nonfluorescent index matching fluid. The prism was made of BK7 glass. The configuration is shown in Fig. 1. The assembly was then placed on a rotary stage that allows excitation and observation at all angles relative to the vertical axis of the center of the prism [4]. All measurements presented were performed in the reverse Kretschmann (RK) configuration unless otherwise noted. This is shown in Fig. 1, where the sample is illuminated with the incident light causing direct excitation of the fluorophores. It is important to note that surface plasmons are not directly created by the excitation so that the angle of incidence is not important. There is another mode of excitation, termed the Kretschmann (KR) configuration. In this configuration, illumination of the system shown in Fig. 1 occurs through
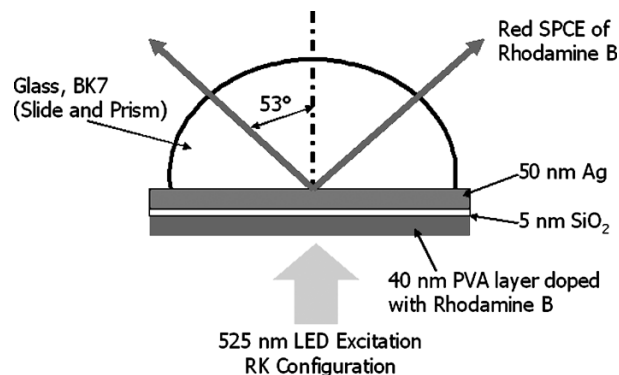

Fig. 1. Setup for the LED excitation of SPCE of rhodamine. In the RK configuration, a 525 nm LED focused with a lens directly excites the sample deposited on the silvered slide. The red, p-polarized rhodamine emission exits the prism at an angle of 53°.

the glass prism at the SPR angle for the excitation wavelength creating surface plasmons. The surface plasmons generate an evanescent field that causes the excitation of the fluorophores. The radiation from the fluorophores is emitted back through the prism at the SPR angle for the emission wavelength.

Excitation of the sample was achieved by focusing a 525 nm LED with a lens. The current through the LED during measurements was 36.2 mA, which is approximately 6 mW of power. The light was filtered with a 550 nm short pass filter to decrease the amount of longer wavelengths propagating from the LED. A polarizer was placed in front of the LED to create s-polarized incident light. This was done to facilitate separation of the excitation and the emission, as SPCE is highly p-polarized. The emission was observed through a 600 nm LWP filter placed in front of a 3 mm diameter liquid light guide (UV–Vis, Oriel). The light guide was covered with a 200 μm vertical slit, and was positioned about 10 cm away from the assembly. The output of the light guide was sent to an 8000 SLM spectrofluorometer. Similar measurements were performed with excitation from a 532 nm laser that was s-polarized to provide a comparison.

RESULTS AND DISCUSSION

Previous work examining the effects of sample thickness on SPCE predicts that the layer spin-coated onto the surface should be approximately 40 nm [7]. For the four-phase system shown in Fig. 1, assuming that the thickness of the sample layer is 40 nm, the reflectivity curve shown in Fig. 2 can be calculated. The reflectivity minimum, corresponding to the SPR angle for the system at
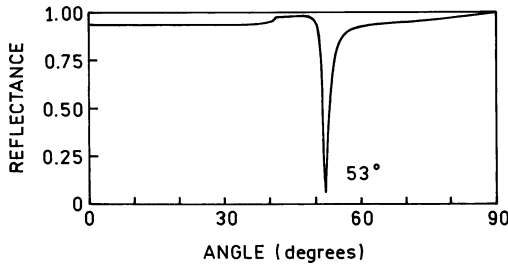
Fig. 2. The reflectivity curves calculated for the four-layer system shown in Fig. 1 at the emission wavelength. The SPR angle for this is 53°.

the emission wavelength, occurs at 53°. The wavevector matching requirements for SPR to occur are similar to those for SPCE, with fractional displacement of the angle due to the evanescent wave [2,26,27]. Therefore, it can be expected that the directional emission will be observed at 53°.

Indeed this was the case. The angular distribution of the SPCE is shown in Fig. 3A. The angle of the emission is the same as the SPR angle calculated for the system at the emission wavelength. The angular distribution for SPCE with laser excitation was also measured. The SPCE angle in this case was 52° as shown in Fig. 3B. The change in the angle is most likely due to a different sample thickness at the excitation point.

It is important to note that the emission from the sample was visible to the eye through a 600 nm LWP filter, as seen in Fig. 4. The light can be seen propagating through the prism (the blurriness) in Fig. 4 (left). Shifting the observation angle away from the plasmon angle causes

the blurriness to disappear because you are no longer observing at the angle of propagation. This is seen in the center of Fig. 4, where the only emission observed is the free space (FS) emission due to direct excitation of the sample. Also shown in Fig. 4 (right) is a time-lapsed photograph showing the excitation and emission of the system.

A comparison of the angular distributions from the different types of excitations shows a broadening of the curve for LED excitation. It is believed that the distribution is not as sharp as that with laser excitation due to the difference in spot size illuminated. The increased area of excitation with the LED due to focusing limitations broadens the distribution because there is a larger amount of fluorophores being excited over a larger area. If the LED was collimated better, it is expected that the angular distribution would become narrower and be nearly indistinguishable from that created with laser excitation.

Next, the spectrum of the emission was examined under various conditions. In order to verify that the sample was exhibiting the phenomenon of SPCE, it is necessary to determine the degree of polarization of the sample and the degree of signal enhancement. The resulting spectra are shown in Fig. 5A. To measure the degree of polarization, a polarizer was placed in front of the light guide. It was oriented to either pass s-polarized or p-polarized light. The results show that the emission from the sample was highly p-polarized. This is expected for SPCE because SPR only occurs for p-polarized incident light. The emission was calculated to have 92% p-polarization.

The FS spectrum due to direct excitation of the sample with the LED was also measured. The light guide was placed at an angle corresponding to the sample side. It is clear from the figure that the intensity gains are great.
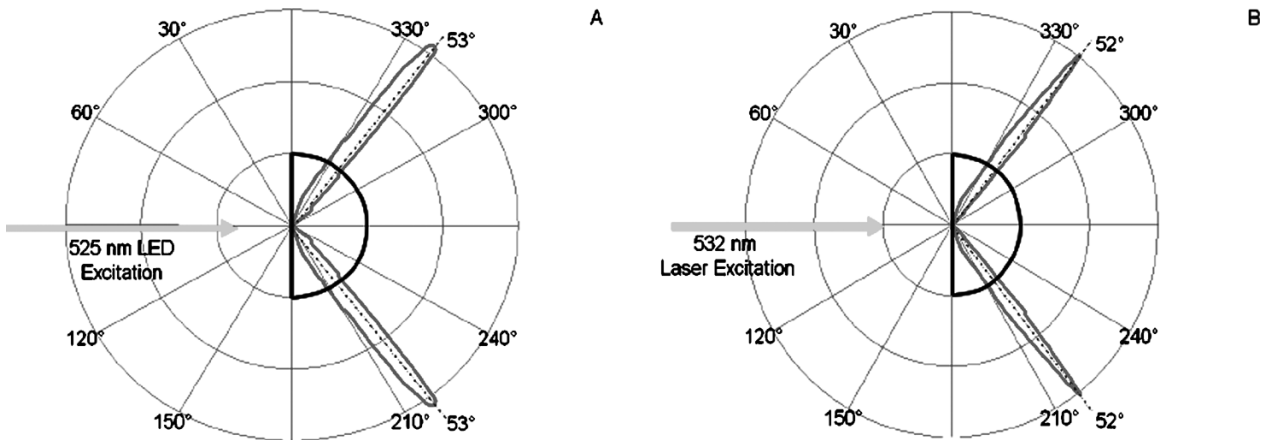

Fig. 3. The angular distribution of the SPCE resulting from RK configuration with LED (A) and laser (B) excitation.
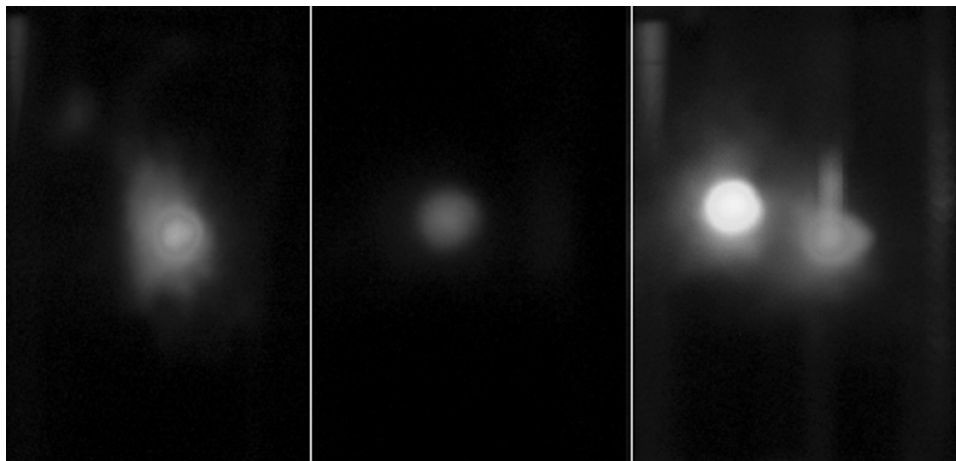
Fig. 4. Left: Photograph of the SPCE from LED excitation as observed with the naked eye through a 550 LWP filter. The blurriness can be attributed to the light emitted at the plasmon angle propagating through the prism. Center: Photograph taken at 10° from the SPCE angle. The emission spot that appears is due to the incident excitation that can be seen through the thin metal layer. Right: Time-lapsed photograph at the SPCE angle. In the beginning no filter was used, which allows observation of the green excitation from the LED. Then, the filter was added resulting in the red from the rhodamine emission.

A comparison of the maximal intensity from the RK configuration to the FS emission shows that there is approximately a 40-fold enhancement of the fluorescence signal. The signal enhancement is a general characteristic of SPCE due to the high collection efficiency, which is a consequence of the directionality of the emission.

Similar measurements were made with laser excitation as shown in Fig. 5B. The emission again showed a high degree of polarization. In this case, it was calculated to be 93% p-polarized as expected for SPCE. The

signal enhancement with the laser was not measured in our case because much experimental work has been done to show that it is present. Published reports have shown enhancements ranging from 3- to 12-fold [4,28]. The increased enhancement observed with the LED compared to these reported values from laser excitation is believed to be due to the broad spectrum (40 nm FWHM) emitted by the LED. This allows the excitation wavelength to better overlap with the excitation maximum of the sample so that the coupled emission would be greater than in the
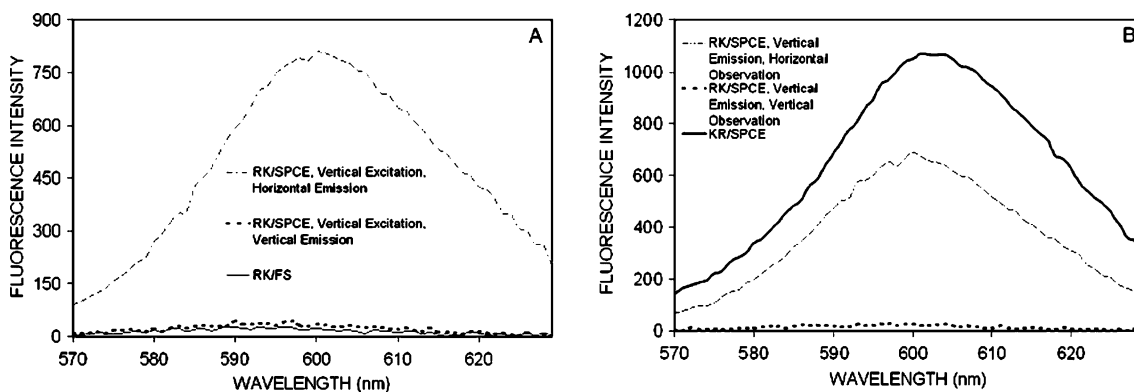

Fig. 5. (A) The emission spectra of rhodamine due to LED excitation measured in the RK/SPCE with the orientation of the polarizers as described. Also, the RK/FS emission spectrum is shown. The emission was highly p-polarized with $I_{VH}/I_{VV} = 24$ giving 92% polarization. (B) The emission spectra of rhodamine due to laser excitation measured in the RK/SPCE with the orientation of the polarizers as described. Also, the spectrum of rhodamine in the KR/SPCE is shown. The emission was highly p-polarized with $I_{VH}/I_{VV} = 27$ giving 93% polarization.

cases where the laser used did not exactly correspond to the maximum.

Also in Fig. 5B, the spectrum due to KR configuration is presented. It should be noted that this type of excitation was not detectable with the LED. This is most likely due to the spot size of the focused light, as well as the broad spectrum produced by the LED (40 nm FWHM) as compared with a laser. Together, these factors make it difficult to have enough light incident at the SPR angle to couple with the plasmons to generate an evanescent field strong enough to create a measurable fluorescence signal. In order to perform the KR mode with LED excitation, the light would have to be collimated using more complex optical equipment than a simple lens which would allow more incident light of the appropriate wavelength to propagate at the necessary angle.

Excitation via the KR mode is important because it is more feasible for use in sensing applications. The ability to excite the sample and collect its emission in a way that provides as little interference as possible is most desirable. The KR mode provides such a scenario where excitation and emission collection can occur from the same direction. It has been reported that the KR mode creates about a 20-fold field enhancement of the incident intensity due to resonance interactions [2,29]. Such an enhancement is shown in Fig. 5B where the KR spectrum has a higher intensity than the RK spectrum. Because of this enhancement, we believe that it is possible to collimate the LED enough to allow for detection of SPCE from KR excitation. We are currently investigating ways to make this possible.

Finally, photobleaching measurements were performed in the RK configuration to compare the effects of the laser and the LED. The data is shown in Fig. 6. Examining this carefully one can see that the laser produced some photobleaching in the first few seconds of illumination, while the LED produced no deterioration at all. After the first few seconds of laser excitation, it appears the dye was stable. This is believed to be caused by the high concentration of the dye used. However, a comparison of the data from the first few seconds leads us to believe LED excitation will not create significant photodeterioration.

The results presented here show that the LED excitation of a fluorophore in close proximity to a thin metal film results in surface plasmon coupled emission. The directionality and polarization of the emission are similar to SPCE measured from laser excitation. The directional emission also showed a great enhancement over the isotropic, free space emission. Normal observation of the fluorescence of the sample would not exhibit these characteristics, suggesting that the emission due to LED excitation is due to plasmon-coupling.
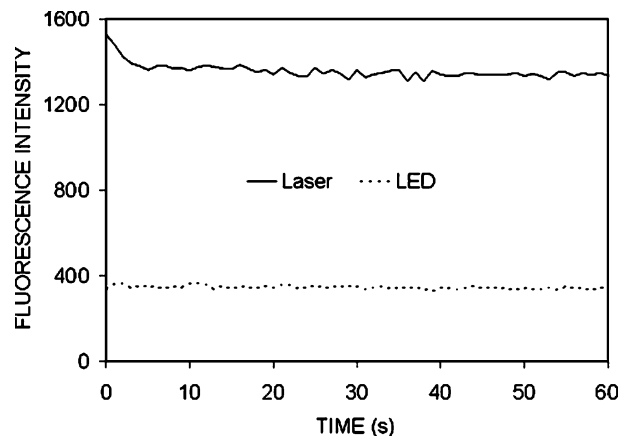

Fig. 6. Comparison of the laser and LED photobleaching illuminated in the RK configuration.

CONCLUSION

The ability to excite SPCE with light-emitting diodes could lead to significant advances in fluorescence spectroscopy. Without the need for enhanced excitation, but only a small LED, handheld, low-cost instruments can be designed using the phenomenon. Coupling the directionality and the visibility of the emission (Fig. 4), one could imagine the detector being a simple photo diode. Instrumentation incorporating SPCE would have far greater sensitivity, leading to unprecedented detection limits, without the high cost of current instrumentation of similar sensitivity. Also, these high-cost instruments are mainly lab-oriented devices, while incorporation of SPCE excited by light-emitting diodes will allow for instrumentation that can be developed to work in the field.

ACKNOWLEDGMENTS

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