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Fluorescence News

Directional Surface Plasmon Coupled Emission

KEY WORDS: Surface plasmon coupled emission; high sensitivity detection; reduced background; spectral discrimination; surface plasmons; evanescent field.

We report a new exciting method for fluorescence detection which promises to increase sensitivity by up to 1000-fold. Our new method depends on the coupling of excited fluorophores with the surface plasmon resonance present in thin metal films, typically silver, gold or aluminium. This new phenomenon, surface plasmon coupled emission (SPCE) occurs for fluorophores 20–250 nm from the metal surface, where emission occurs over a narrow angular distribution, converting normally isotropic emission into easily collected directional emission. The interaction is *independent* on the mode of excitation, i.e. does not require evanescent wave or surface plasmon excitation. With typical optical components, the collection efficiency is 1% or less, however our new approach promises to couple up to 50% of the emission from unoriented and appropriately distanced fluorophores. We believe our new findings offer both unique and exciting possibilities for high sensitivity fluorescence detection, as distal fluorescence (autofluorescence) from fluorophores or species from the metal surface only weakly couples. In addition SPCE is highly polarised and autofluorescence can be further discriminated against by collecting only the polarised component or the light emanating with the appropriate angle. Further, different emission wavelengths couple at different angles allowing spectral discrimination without additional optics.

In our preliminary studies of SPCE a continuous 50 nm thick silver film on a glass substrate was spin coated with varying thicknesses of sulforhodamine 101 (S101) doped PVA [1]. The spin-coated glass slides were attached to a hemi-cylindrical prism made of BK7 glass, Fig. 1. This combined sample was positioned on a precise rotary stage, Fig. 2, which allows excitation and observation at any desired angle relative to the vertical axis along the cylinder. Two modes of excitation were considered in our studies, Fig. 3. The sample could be excited through the prism. In this case the incident light was completely reflected at all angles except when the incident light angle equalled the surface plasmon angle, θ_{sp}. For incident angles near the θ_{sp} there exists an evanescent wave in the air sample side, distal from the incident light. This envanescent field, which is enhanced about 20-fold by the resonance interaction [2,3], extends about 200 nm into the air or sample. This mode of illumination is typically called the Kretschmann (KR) configuration, Fig. 3. Alternatively the sample can also be excited from the air or sample side which has a refractive index lower than that of the prism. Here surface plasmons are not excited [1]. While the angle of incidence is not important in this reverse Kretschmann (RK) configuration, in our studies we employed normal incidence.

We have found that the mode of excitation does not matter for SPCE [1,4–6]. That is, an excited fluorophore should couple with the surface plasmons whether it is excited by the surface plasmon evanescent field or directly using the reverse Kretschmann configuration. Figure 4 shows the dependence of the emission intensity on observation angle with RK excitation, noting that the incident field cannot induce surface plasmons [1]. On the prism side (Back side B) of the sample, the emission is very sharply distributed at $\pm 47^\circ$ or $\pm 50^\circ$ from the normal axis for both the 15 (Top) and 30 nm (Bottom) films respectively. This small difference in angle is due to the thickness of the PVA film. The intensity observed on the front (F) side of the sample was much lower and not sharply distributed at any particular angle, Fig. 4. Higher emission intensity is observed on the front side of the thicker sample (Bottom), which is consistent with lower efficiency coupling into the surface plasmon for fluorophores more distal from the metal surface. In all our experiments

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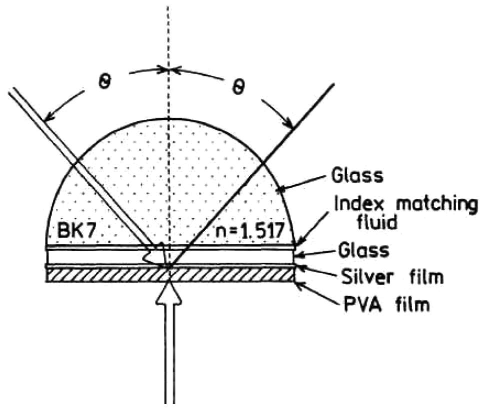

Fig. 1. Configuration of the hemi-cylinder and spin-coated PVA-fluorophore slide. Adopted from [1].

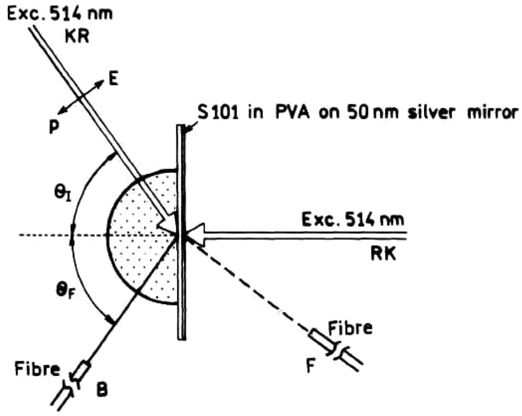

Fig. 3. Experimental geometry for the measurement of free space emission (**F**), and SPCE (**B**), with the Kretschmann (**KR**) and reverse Kretschmann (**RK**) configurations. Adopted from [1].

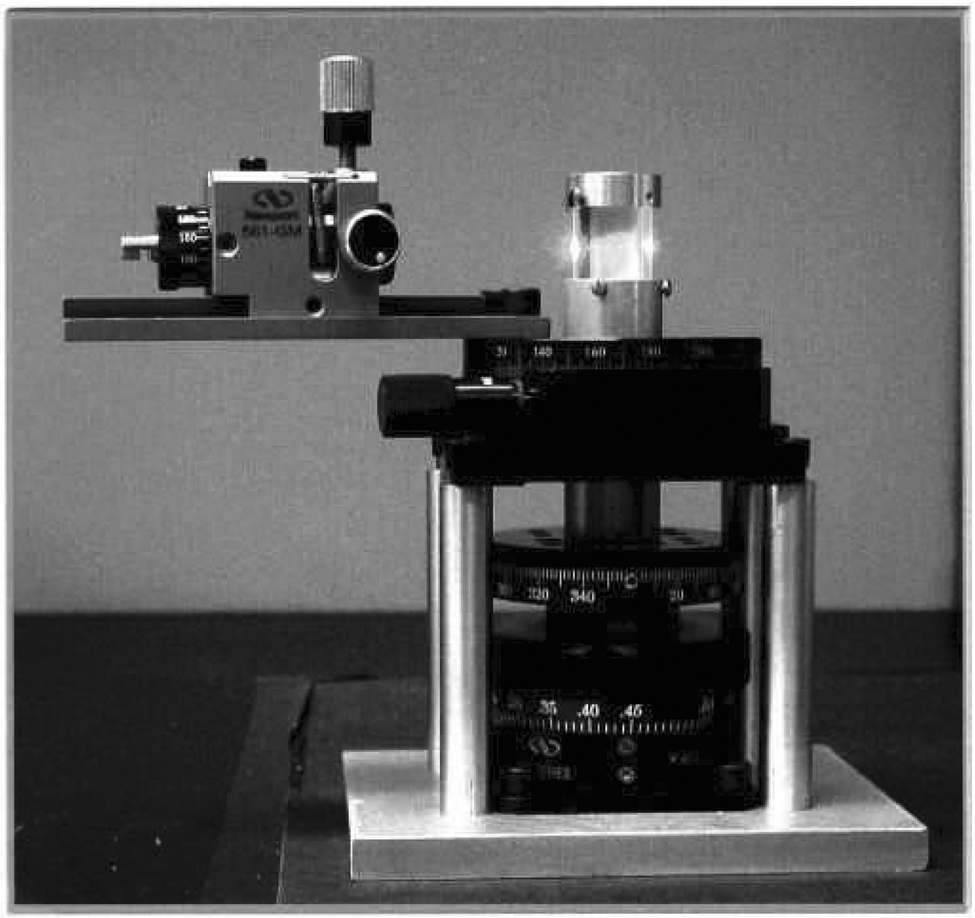

Fig. 2. Rotation stage and sample holder for directional excitation and emission measurements. For excitation we used the 514 nm output of a mode locked argon ion laser, 76 MHz repetition rate. Scattered light at 514 nm was suppressed by observation through a holographic supernotch-plus filter. Emission intensities were observed through a long-wave-pass filter LWP 550 in addition to the notch filter. Observation of the emission was performed with a 3-mm diameter fibre bundle, covered with a 200 μm vertical slit, positioned about 15 cm from the sample. This corresponds to an acceptance angle below 0.1°. Adopted from [1].

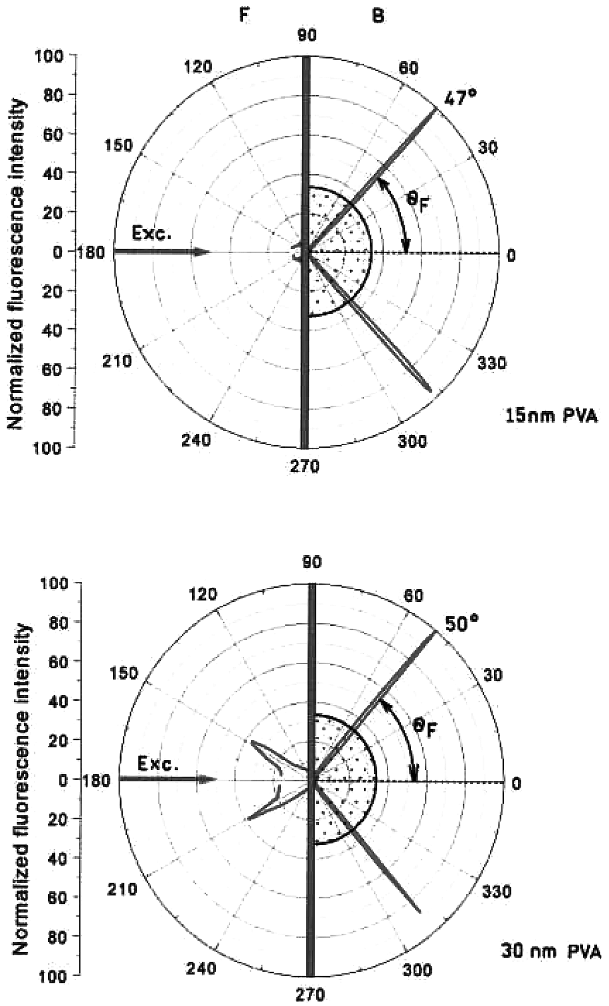

Fig. 4. Angular dependence of S101 emission excited using the reverse Kretschmann configuration. The PVA thickness was approximately 15 nm (Top) and 30 nm (Bottom). Adopted from [1].

scattered light was carefully examined and rejected. We additionally examined the polarisation of SPCE and found that the emission is polarised in the plane of incidence (*p*-polarised) irrespective of the polarisation of the excitation. This polarisation of the directional emission is an interesting find and proves that it is due to coupling of the surface plasmons, and that the polarization of the SPCE is independent of the polarisation of the normal incidence excitation. This suggests that the emission dipoles parallel to the plane of incidence couple into the surface plasmon, and dipoles perpendicular to the plane of incidence do not result in SPCE, or at least display only very weak coupling [1,4]. Figure 5 shows the emission for S101 in PVA observed with a hemi-spherical prism and RK excitation, where the hemi-spherical prism allows for the SPCE cone to be observed.

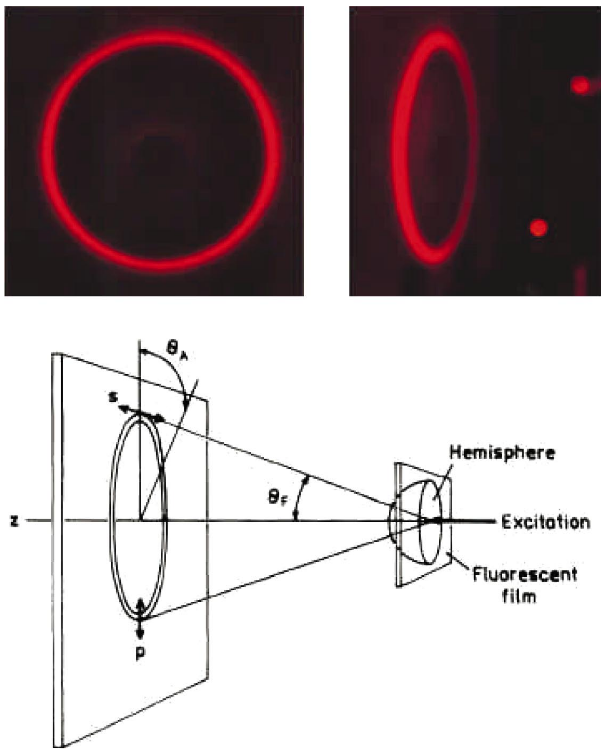

Fig. 5. Emission for S101 in PVA observed with a hemi-spherical prism and RK excitation (Top) and the cone of emission with a hemi-spherical prism (Bottom). The emission was incident on tracing paper and photographed through a LWP 550 filter. Adopted from [1].

Finally we have found that SPCE strongly depends on wavelength. This suggests that fluorophores with different emission maxima will display SPCE at different angles. Subsequently Fig. 6 shows the SPCE from a mixture of fluorophores using RK excitation and a hemi-spherical prism, with 532 nm excitation. We recorded the emission spectra at different observation angles where the spectra are clearly distinct at each angle, with the shorter wavelengths occurring for larger angles [4]. This remarkable find suggests the potential for multi-fluorophore surface assays featuring the intrinsic spectral resolution of SPCE.

CLOSING REMARKS

In this brief article we have shown some of our recent finings for Surface Plasmon Coupled Emission. As we have shown, SPCE offers numerous advantages for high-sensitivity detection in a potentially wide range of formats, e.g.

- Surface bound assays, e.g. immunoassays or DNA arrays. The extent of background interference for these assays will be reduced due to the weaker

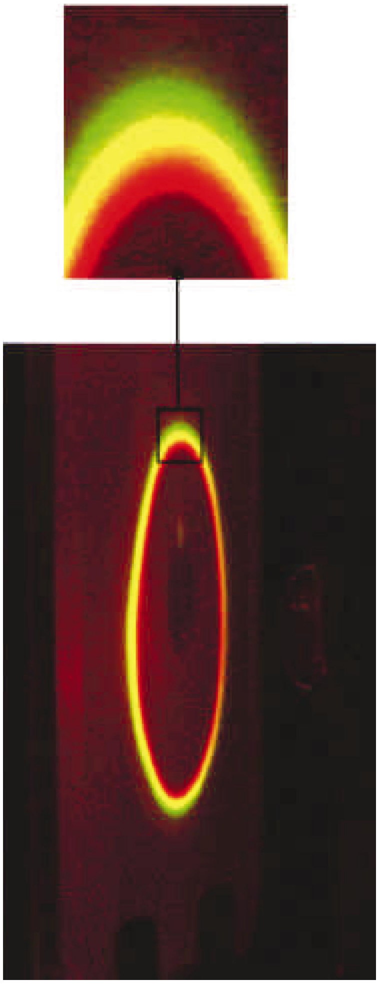

Fig. 6. Photograph of SPCE from a mixture of S101, Rhodamine 123 and Pyridine 2, using RK excitation and a hemi-spherical prism. Initially the fluorophore concentrations were optimized to give similar free space fluorescence intensities. Adopted from [1].

coupling of the more distal fluorophores. The illumination intensity can also be decreased due to the enhanced incident field, which may reduce the autofluorescence from all the optical components, including the glass or plastic substrates.

- SPCE may find applications in High Throughput Screening. It would be relatively simple to coat microlitre plates with silver or even the less reactive gold, which we have recently found similar effects for [7].
- SPCE may also facilitate Single Molecule Detection, SMD. In SMD the total signal level is often limited by the collection efficiency and the autofluorescence from the sample/s. The use of SPCE could increase the efficiency of light collection as well as reducing background fluorescence.
- Combining SPCE with the effects of small metallic sub-wavelength sized particles. These particles are known to increase the quantum, yields and photostabilities of fluorophores within 10 nm [8–10]. Thus appropriately positioned fluorophores should display a dramatic increase in intensity due to the interactions of the metallic particles and directional emission at the surface plasmon angle of the emission.

Further work is underway in our laboratories and will be reported in due course.

REFERENCES

1. I. Gryczynski, J. Malicka, Z. Gryczynski, and J. R. Lakowicz (in press). Radiative decay engineering 4. Experimental studies of surface plasmon-coupled directional emission. *Anal. Biochem.*
2. T. Liebermann and W. Knoll (2000). Surface Plasmon field-enhanced fluorescence spectroscopy. *Colloid Surf.* **171**, 115–130.
3. K. Kurihara and K. Suzuki (2002). Theoretical understanding of an absorption-based surface plasmon resonance sensor based on Kretschmann theory. *Anal. Chem.* **74**, 696–701.
4. J. R. Lakowicz (in press). Radiative decay engineering 3: Surface plasmon-coupled directional emission. *Anal. Biochem.*
5. J. Malicka, I. Gryczynski, Z. Gryczynski, and J. R. Lakowicz (2003). DNA hybridization using surface plasmon coupled emission. *Anal. Chem.* **75**(23), 6629–6633.
6. J. R. Lakowicz, J. Malicka, I. Gryczynski, and Z. Gryczynski (2003). Directional surface plasmon-coupled emission: A new method for high sensitivity detection. *Biochem. Biophys. Res. Comm.* **307**, 435–439.
7. I. Gryczynski, J. Malicka, Z. Gryczynski, and J. R. Lakowicz (submitted). Surface plasmon-coupled mission using gold films. *J. Phys. Chem. B.*
8. C. D. Geddes and J. R. Lakowicz (2002). Metal enhanced fluorescence. *J. Fluoresc.* **12**(2), 121–129.
9. J. R. Lakowicz (2001). Radiative decay Engineering: Biophysical and biomedical applications. *Anal. Biochem.* **298**, 1–24.
10. J. R. Lakowicz, Y. Shen, S. D'Auria, J. Malicka, J. Fang, Z. Gryczynski, and I. Gryczynski (2002). Radiative decay engineering 2. Effects of silver island films on fluorescence intensity Lifetimes and Resonance energy transfer. *Anal. Biochem.* **301**, 261–277.

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