

Metal-enhanced fluorescence: Surface plasmons can radiate a fluorophore's structured emission

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In this letter, the authors report the observation of metal-enhanced structured fluorescence emission. Perylene in close proximity to silver island films (SiFs) enhances the structured fluorescence emission intensity. In this regard, an approximately two-fold higher perylene fluorescence intensity was observed from SiFs as compared to a glass control sample, containing no silver nanoparticles. The findings strongly suggest that surface plasmons can radiate a fluorophore's vibrational structure. This observation is helpful in the authors' understanding, not only for studying the interactions between plasmons and lumophores but also for their laboratories' continued efforts to develop a unified plasmon-lumophore theory. © 2007 American Institute of Physics.

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Fluorescence is the dominant technique in medical diagnostics and biotechnology today. However, the detection limit is usually limited by the quantum yield of the fluorophore (label), the autofluorescence of the sample, and the photostability of the fluorophores. In this regard, there has been a recent explosion in the use of metallic nanostructures to favorably modify the spectral properties of fluorophores and to alleviate some of their more classical photophysical constraints.¹⁻⁴ The use of fluorophore-metal interactions has been termed metal-enhanced fluorescence¹ (MEF) or radiative decay engineering (RDE) by us.²⁻⁴

In recent years, our laboratories have both studied and demonstrated many applications of MEF.⁵⁻⁷ These have included the increased detectability and photostability of fluorophores,⁵ improved DNA detection,⁸ release of self-quenched fluorescence of overlabeled proteins,⁹ enhanced wavelength-ratiometric sensing,¹⁰ and application of metallic surfaces to amplified assay detection.¹¹ Our laboratories' current interpretation of MEF has been explained by a model whereby nonradiative energy transfer occurs from excited distal fluorophores to the surface plasmon electrons in non-continuous films (Fig. 1 middle), in essence a fluorophore induced mirror dipole in the metal. The surface plasmons in turn radiate the emission of the coupling fluorophores.¹² This explanation has been facilitated by our recent observation of surface plasmon coupled emission (SPCE),¹³ whereby fluorophores distal to a continuous metallic film can directionally radiate fluorophore emission at a unique angle from the back of the film. This interpretation has also led to the development of plasmon coupled enhanced chemiluminescence¹⁴ and plasmon coupled phosphorescence.¹⁵ However, our current explanation of plasmon-lumophore interactions is subtly

different than our early reports, where we postulated that it was the fluorophore itself that radiated (Fig. 1 top), its photophysical properties thought to be modified by a resonance interaction in close proximity to surface plasmons.¹

In addition, nearly all previous MEF studies exclusively focused on unstructured fluorescence emission,¹⁻¹⁰ where ir-

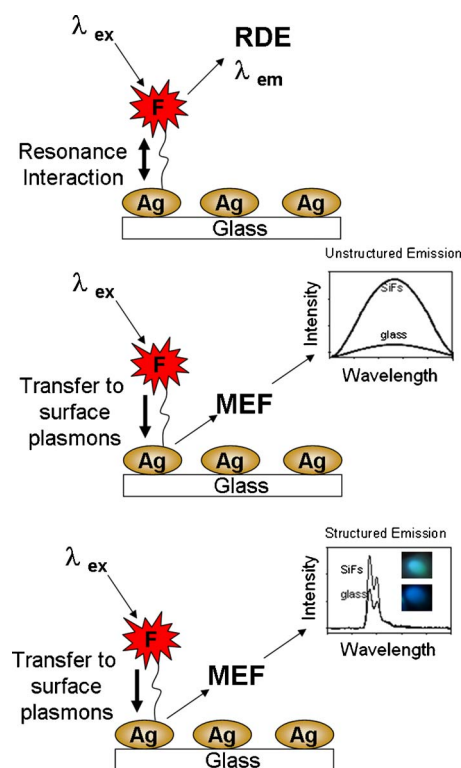


FIG. 1. (Color online) Original interpretation of MEF, i.e., RDE (top), current interpretation (middle), and plasmon coupled structured emission (bottom). F, fluorophore; RDE, radiative decay engineering; MEF, metal-enhanced fluorescence.

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radiation with a single or wide spectrum of wavelengths generates an entire range of allowed transitions that populate the various vibrational energy levels. Subsequently, we have now questioned whether surface plasmons can in fact radiate a fluorophore's vibrational structure. For structured emission, the transitions from the lowest vibrational level of an excited state to several vibrational levels of the ground state usually have a much higher degree of probability than others, and when combined, constitute the structured emission spectrum of that molecule.¹⁴ Fine vibrational structure can allow a detailed analysis of electronic and chemical states, of vibronic coupling, and of local vibrational property. Amplified vibronic structure may well yield extra information about intra- and intermolecular interactions. In addition, it can provide a very detailed fingerprint for the discrimination of chemical substances with subtle differences, or in slightly different physical states and surroundings. In this letter we subsequently report our observations of perylene in close proximity to SiFs, which has both a well-known structured absorbance and fluorescence emission. We have observed the enhanced absorption of perylene and the enhanced emission (which is approximately two-fold brighter) from SiFs as compared to a glass control substrate. Furthermore, a shorter perylene fluorescence lifetime, when in close proximity to silver nanostructures, is in complete agreement with other reports for metal-enhanced unstructured fluorescence.⁵⁻⁷

In this study, SiFs were prepared as we have previously published.¹¹ 300 μl of perylene ($1.0 \times 10^{-4} M$) in cyclohexane was sandwiched between both the glass slides and the silver island films. Figure 2 top inset shows the experimental sample geometry.

Figure 2 top shows the absorption spectra of perylene from between the SiFs and from glass. SiFs and glass without perylene were used as reference backgrounds for the perylene absorption measurements, respectively. Perylene on glass exhibits a structured absorbance spectrum which includes three separate bands. The wavelength of maximum absorption at 436 nm represents the transition from the ground state to the lowest vibrational state in the excited state which has the highest degree of emission probability. The peaks at 407 and 387 nm are the transitions from the ground state to the second and third vibrational levels of the excited state, respectively.¹⁶ When perylene is in close proximity to SiFs, the absorption bands at 436, 407, and 387 nm increased by approximately four, three, and two times, respectively. For unstructured absorption spectra, similar enhancements have also been previously observed,^{17,18} giving us some degree of confidence in our observations and indeed interpretations. When a lumophore is placed near a metal, there is often a very strong net absorption effect, a result of the coupling of the molecular dipoles with the localized electromagnetic field of the metallic particle's surface plasmon resonance.⁶ In essence, conducting metallic particles can modify the free space absorption condition in ways that increase the photonic mode density and incident electric field felt by a lumophore.² In addition, the enhanced absorption phenomenon can directly result in surface enhanced luminescence phenomena, such as MEF,¹⁻¹⁰ metal-enhanced phosphorescence,¹⁵ and metal-enhanced S_2 emission,¹⁷ phenomena described by our groups. This is the first observation that a structured absorbance spectrum is also enhanced by surface plasmons.

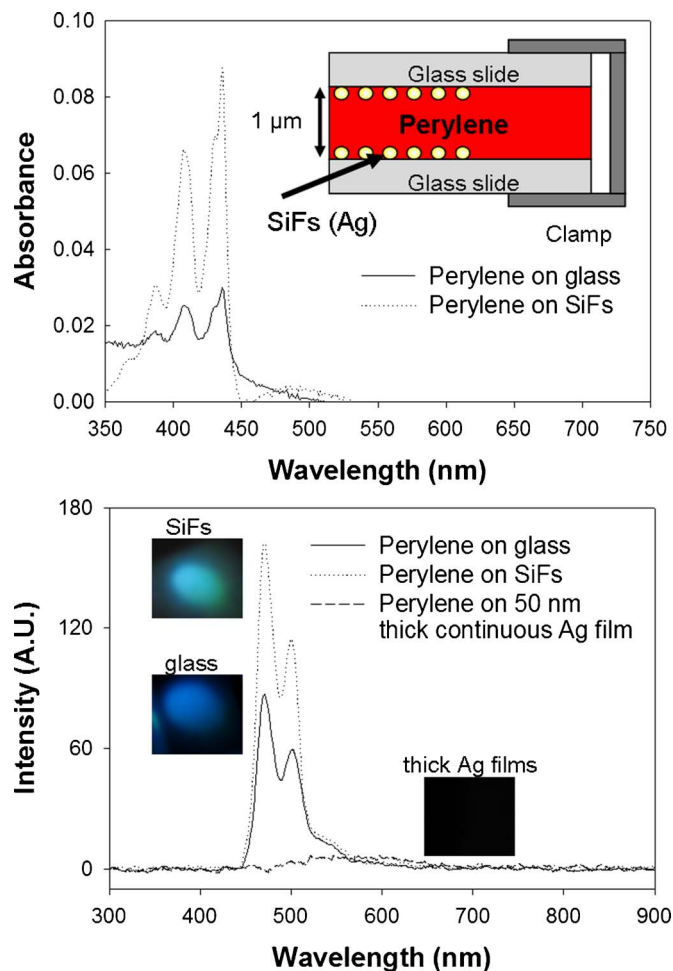


FIG. 2. (Color online) Absorption spectra of perylene sandwiched between to silvered and unsilvered slides, respectively (top). Glass and SiFs without perylene were used as blanks for the absorbance measurements. Sandwich experimental geometry (top inset). Fluorescence emission spectra of perylene sandwiched between two silvered and unsilvered slides, and 50 nm thick continuous Ag films, respectively (bottom). Photographs showing the respective emission. Solvent: cyclohexane.

Figure 2 bottom shows the structured fluorescence emission spectra of perylene on SiFs and glass. Perylene shows significant structure (Fig. 2 bottom) due to transitions from the lowest vibrational level of the excited S_1 state to several vibrational levels of the ground state, those having a much higher degree of probability than others. The energy spacing between the various vibronic energy levels, which is about 1500 cm^{-1} , is very similar to the absorbance spectra band separation. It was also observed that the enhanced structured fluorescence intensity was approximately two-fold brighter from the silver, as compared to glass. In this regard, it should be noted that the true metal-enhanced fluorescence enhancement factor is actually approximately 50-fold. This is because the MEF phenomenon is distance dependent, where with a sample thickness of $\sim 1 \mu\text{m}$ and an enhanced interaction region $< 20 \text{ nm}$,⁷ only 4% of the sample is within the MEF enhancement region, as depicted by Fig. 2 top inset. In addition, MEF is also dependent on the size and shape of the metallic nanoparticles, as those support surface plasmons.⁵⁻⁷ As a control sample, perylene emission from a continuous strip of silver was tested and is observed to be dark (Fig. 2 bottom), i.e., no emission. It is known that continuous metallic surfaces traditionally quench close proximity

TABLE I. Fluorescence intensity decay analysis. τ , mean lifetime; $\langle\tau\rangle$, amplitude-weighted lifetime. SiFs, silver island films. $\alpha_1 + \alpha_2 + \alpha_3 = 100$; α_n , amplitudes.

	τ_1 (ns)	α_1 (%)	τ_2 (ns)	α_2 (%)	τ_3 (ns)	α_3 (%)	$\langle\tau\rangle$ (ns)	τ (ns)	χ^2
Perylene on glass	6.49	3.579	3.078	74.92	0.991	21.49	2.751	3.204	1.16
Perylene on SiFs	5.33	10.76	1.377	40.31	0.278	48.93	1.264	3.051	0.99

luminescence.¹⁴ It is also known that the generation of surface plasmons in continuous metallic strips occurs only under certain unique conditions, as compared to the simplicity of creating surface plasmons in subwavelength sized nanoparticles.^{4,13} This observation strongly suggests that the enhanced vibronic emission is plasmon enhanced.

In addition, we studied the intensity decays of perylene in close proximity to SiFs. The respective lifetimes shown in Table I were calculated from those decays, using nonlinear least squares impulse deconvolution analysis. From Table I, we can see both a reduced mean lifetime ($\tau_{\text{mean on SiFs}} = 3.051$ ns) and amplitude-weighted lifetime ($\langle\tau\rangle_{\text{on SiFs}} = 1.264$ ns) for fluorophores near silver as compared to the glass control sample ($\tau_{\text{mean on glass}} = 3.204$ ns, $\langle\tau\rangle_{\text{on glass}} = 2.751$ ns). In fact, a highly efficient structured emission coupling to surface plasmons, where the plasmons efficiently and quickly radiate the coupled emission, would be expected to result in a reduced fluorophore lifetime and enhanced observed structured emission intensity. These findings are consistent with our previously reported findings for nanosecond decay time fluorophores with *unstructured* emission sandwiched between identical silver nanostructures, similarly suggesting that the radiating plasmon model¹² is a suitable description of the enhancement mechanism¹² (Fig. 1 bottom).

In conclusion we report the first observation of surface plasmons radiating a fluorophore's vibrational structure (Fig. 1 bottom). Perylene in close proximity to silver island films can undergo enhanced fluorescence; a twofold increase (actual enhancement is approximately 50-fold) was observed as compared to an identical control sample containing no silver. At first this observation may seem surprising, as the radiating plasmon model is underpinned by a mirror dipole effect.¹² This therefore implies that vibrational transitions are also mirrored on nanoparticles by surface plasmons (Fig. 1 bottom). Given that vibrational relaxation is an exceedingly quick process, then our findings imply that transfer to plasmons occurs on an even quicker time-scale, before structured

emission. This observation is helpful in our understanding, not only for studying the interactions between plasmons and lumophores but also for our laboratories' continued efforts to develop a unified plasmon-lumophore theory.

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