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ORIGINAL PAPER

Low Temperature Metal-Enhanced Fluorescence

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Abstract In this short letter, we describe the effects of low temperature on the Metal-Enhanced Fluorescence (MEF) phenomenon. Fluorophores close to Silver Island Films (SiFs) show on average two- to ten-fold enhancements in their fluorescence signatures at room temperature. However, at 77 K, we have observed that MEF is even more pronounced as compared to an identical glass control sample. We also demonstrate that the further enhancements in MEF occur at low temperature over a range of visible wavelengths for different fluorophores, for both SiFs and 20 nm surface deposited gold colloids.

Keywords Metal-Enhanced fluorescence · Radiative decay engineering · Surface enhanced fluorescence · Plasmon controlled fluorescence · Radiating plasmons · Low temperature · Plasmon enhanced luminescence · Low temperature plasmonics

Since the early observations of fluorophore–metal interactions [1], there has been a plethora of literature describing the photophysical effects of fluorophores in close proximity to metallic nanostructures [2–5]. In recent years our laboratory has contributed significantly to this fundamental understanding[6], including the first use and introduction of the term "Metal-Enhanced Fluorescence (MEF)" [6]. Previously, we described metal-enhanced fluorescence as due to the resonance interaction of a fluorophore with a plasmon resonant particle, giving rise to a modification of a fluorophore's intrinsic radiative decay rate [6] Fig. 1, top. This description was fueled by earlier workers who had shown increases in fluorescence emission, coupled with a simultaneous drop in radiative lifetime [3, 6–8]. Accordingly, in classical fluorescence, these two effects could only be described by a change in a fluorophore's intrinsic radiative decay rate [6, 8]. In addition, other workers had shown that the enhancement effect was distance dependent [4] Fig. 1 top and middle, with close proximity to metal resulting in fluorescence quenching [6, 8], a consequence of phase induced radiative rate suppression [9] or "damping".

However, in the last 5 years, our understanding of metal-enhanced fluorescence has changed from one originally thought to involve a resonance interaction between the excited fluorophore and surface plasmons, which modifies a fluorophore's radiative decay rate, to one whereby fluorophore dipoles non-radiatively couple to surface plasmons, which in turn, radiate efficiently, the spectral properties of the coupled luminophore, (Fig. 1, bottom) [10]. This description has led to reports of Metal-Enhanced Chemiluminescence [11], Phosphorescence [12], S_2 emission [13] and even a report of structured metal-enhanced emission, [14] to name but just a few. Our recent description of MEF, has been postulated as a result of both ours and others observations of Surface Plasmon Coupled Fluorescence (SPCF), where surface plasmons directionally radiate fluorophore coupled *p*-polarized emission through the back of a continuous metallic film [15, 16]. Subsequently, the Radiating Plasmon Model has been both described and demonstrated experimentally [17, 18]. In this model, metal-enhanced fluorescence is attributed to the scattering portion of the extinction spectrum for metallic particles, i.e. a fluorophore couples to that scattering mode. Given that the absorption component dominates the

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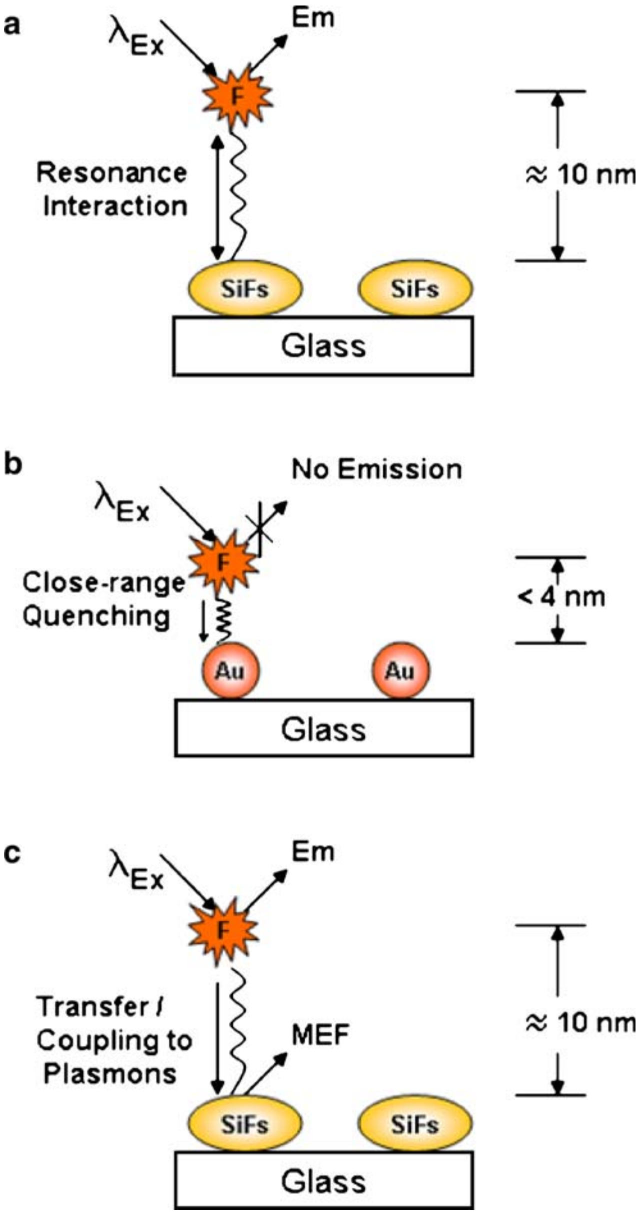


Fig. 1 Graphical representation of original interpretation of metal-enhanced fluorescence (**a**), close range metal quenching of fluorescence (**b**) and current interpretation for metal-enhanced fluorescence (**c**). *F* Fluorophore, *MEF* metal-enhanced fluorescence, Au small gold nanoparticles, *SiFs* Silver Island Films

extinction spectrum for small nanoparticles, then this reasoning has been used to explain fluorescence quenching for both small gold and silver nanostructures [17]. Conversely, larger particles have a more substantial scattering component to their extinction spectrum, which accounts for metal-enhanced fluorescence [17, 18]. In this regard, we have recently seen the observation of MEF from gold substrates, which have been attributed to MEF as compared to changes in absorption cross-section [19]. In this recent paper, 200 nm gold colloids are shown to notably enhance fluorescence signatures in an angular dependent fashion [19]. In all of the reports on MEF today for both silver and gold [8–19], all studies have been conducted at room temperature and no consideration for low temperature and its effect on MEF have been reported.

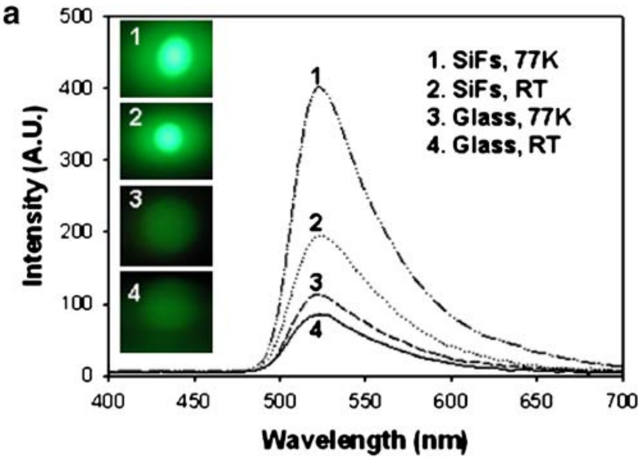
The optical properties of both gold and silver nanoparticles are notably different from the respective bulk metal. As the size of a particle decreases, then the conductance electrons begin to interact at the boundary of nanoparticles [20]. The displacement of the conductance electrons by external electromagnetic energy, induces surface polarization charges, that in turn, result in a restoring force within the metal. At a unique set of frequencies the restoring force results in a characteristic surface oscillation known as the Surface Plasmon Resonance (SPR). In addition to nanoparticle geometry, it is also known that the optical properties of nanoparticles are determined by the electronic structure of the metal, which determines the metal's dielectric function [20]. In modeling the absorption spectra of nanoparticles, the identification of an appropriate dielectric function is crucial [21], some workers using a "damped Drude model", which utilizes a free electron metal with damping. Other workers have modeled metals as Lorentz Oscillators [21], where the silver not only displays free electron behavior but exhibits bound electron effects related to interband transitions [20, 21]. With Plasmon resonant energies well below the interband transitions both silver and gold nanoparticles can typically be treated as free-electron systems, whose optical properties are determined by the conduction band electrons, with only a consistent real background polarizability associated with the inner core electrons [21].

In this letter we have subsequently studied the influence of temperature on the metal-enhanced fluorescence phenomenon [10], for both silver and gold nanoparticles. Our results show that the MEF phenomenon is significantly more pronounced at lower temperature as compared to identical control samples at room temperature. In addition, we have studied the effect for fluorophores whose emission spectra spans the visible range using both gold and silver nanoparticles. Given that no changes in both the silver and gold plasmon absorption spectra are observed from room temperature to 77 K, then we subsequently speculate that at low temperature, a reduction in fluorophore damping, which is typically observed close-to-metal [7, 9, 20, 21], facilitates a further enhanced metal-enhanced fluorescence. Noting again that no change in the SPR is observed at low temperature, coupled with the strong control samples used, then the changes we observe in MEF are further attributed to the changes in the metal, and not the fluorophores, specifically, differences in the non-free electron component of the dielectric function of the metal.

In this study, Silver Island Films (SiFs) were prepared as we have previously published [22]. Typically, SiFs are 4 nm in height and 20 nm in length with a 40% surface coverage on the glass substrate. Further details showing the AFM image of SiFs can be found in previous publications [22, 23]. Low temperature experiments are performed as follows: 300 μL of a fluorophore (Acridine, Acridine orange, Rhodamine B, Cresyl Violet, Nile Blue) (50 uM) in ethanol solution was trapped in a sandwich format between the glass/quartz slides, SiFs and glass/quartz slides containing 20 nm gold nanoparticles completely covering the surfaces, respectively. The glass, quartz, SiFs and gold surfaces were placed in liquid nitrogen for 2 min and used for the low temperature (77 K) fluorescence measurements. Fluorescence measurements were carried out by placing the sample on a sample holder and fluorescence spectra collected using a HD2000 Ocean Optics fluorometer.

Figure 2, top shows the fluorescence spectra of Acridine Orange at room temperature (RT) and 77 K, from both glass and silvered glass substrates. As one can see, the emission spectrum for Acridine Orange is much more intense from SiFs as compared to the glass control sample, an identical sample, but containing no silver nanoparticles. Interestingly, the enhancement factor at RT was ~2.3 as compared to ~3.6 from the same sample at 77 K (Fig. 2, bottom), where the enhancement factor is the emission intensity from silver divided by glass at the respective temperature. Clearly, low temperature results in further enhancement in the fluorescence emission from fluorophores in close proximity to silver nanoparticles. The enhancement factor increased for both maximum intensity enhancement values as well as the integrated area ratios (Fig. 2, bottom in brackets). Interestingly, only slight line narrowing was observed for the samples at 77 K, approximately 4 nm FWHM. Figure 2, top insert also shows the emission enhancement visually at 77 K as compared to the glass control, c.f. photographs and curves 1 and 3, respectively. For all of these samples shown in Fig. 2, the excitation and emission conditions were identical, the only differences being the nature of the sample, i.e. glass or silver, and the temperature.

It is well known that silver nanostructures can alter the absorption properties of fluorophores [6, 8] in essence modifying the excitation rate of fluorophores [6, 8]. These modifications in free space excitation conditions can lend to increased emission intensities and decreased fluorophore photostabilities [6, 8]. Here, no changes in the absorption spectra were observed for the fluorophores sandwiched between the metal, indicating this is not effective here. For a given silvered or glass sample, the excitation conditions are otherwise *identical,* excluding any excitation rate explanations to the enhancement factors between both room and low temperature samples. We have also studied the effect of temperature on fluorophore properties and the subsequent effects on MEF. Our studies show that there is no quantum yield dependence of the fluorophore for further low temperature MEF enhancement as is classically observed for MEF, suggesting that modifications in the fluorophore's radiative / non-radiative rates upon cooling, does not explain the low temperature MEF observations. Therefore, the effects observed are though to be fundamentally due to changes in the dielectric function of the metal and its subsequent effect on MEF, i.e. fluorophore-plasmon coupling and scattering. It should be noted the fluorophore is common to all samples, but its photophysical behavior at low temperature and room temperature on SiFs is notably different, c.f. curves 1 and 3 in Fig. 2, top.



b

Em @ 521 nm	Fluorescence	
	FWHM	Enhancement factor (integrated area ratio)
Acridine orange glass slides, RT	58 nm	-
Acridine orange glass slides, 77K	59nm	-
Acridine orange SiFs, RT	60 nm	2.27 (1.78)
Acridine orange SiFs, 77K	56 nm	3.60 (2.65)

Fig. 2 Fluorescence spectra (**a**) and subsequent spectral data (**b**) for Acridine Orange at RT and 77 K from both glass and silvered slides. The enhancement factor was calculated as the "peak" emission intensity ratio SiFs/Glass. The integrated area ratio is the ratio of the area under the fluorescence spectra on SiFs and glass, respectively. *FWHM* Full-width half-maximum

Since MEF is related to surface plasmons' ability to scatter light according the RPM model [17], and silver and gold absorb and scatter light at different wavelengths and efficiencies [24], we questioned whether low temperature MEF would be dependent on the type of the metal and the wavelength of fluorophores used. In this regard, we studied a range of fluorophores on SiFs and gold nanoparticles with different emission wavelength maxima, using experimental geometries identical to that employed for Acridine Orange.

Figure 3, top, shows the fluorescence enhancement factor versus different maximum emission wavelengths for various dyes on SiFs. The absorption spectrum of SiFs is also given in Fig. 3, top, to compare the enhancement factors with respect to absorption of light by silver. Over the wavelength range ~ 400–660 nm, we observed five different fluorophores whose silver enhancement factor was greater at low temperature as compared to room temperature. We did not find any correlation between the enhancement factors and the emission wavelength nor with any obvious relationship to the plasmon absorption of the silver.

Finally, it is well known that small gold nanoparticles typically quench fluorescence [25–27]. This quenching is known to be a consequence of the dominant contribution of the absorption component, to the extinction spectra, of the nanoparticles [17, 19], i.e. energy transfer quenching with a d^{-4} distance dependence [26]. We subsequently questioned whether low temperatures would *un-quench* fluorescence, especially for fluorophores whose emission maxima is similar to the SPR of the nanoparticles. Using the same five fluorophores, we observed increases in MEF at low temperature as compared to identical samples at room temperature as shown in Fig. 3, bottom. For Acridine, *an additional* approximately four-fold enhancement was observed at low temperature as compared to room temperature. Interestingly, greater enhancements were observed at low temperature for the same fluorophores, which also showed notable enhancements for silver, although the net enhancement factors were generally smaller from gold than silver. Similar to silver, no emission wavelength or quantum yield dependence of enhancement was observed, or could be correlated with gold plasmon resonance. However, for both the SiFs and 20 nm Gold colloid deposited films, all samples showed further MEF increases at low temperature as compared to room temperature. This finding suggests that the changes in the dielectric functions of both metals are similar at low temperature, as are there subsequent influences on MEF. Consistent with our findings are the numerous reports of small nanoparticles quenching fluorescence, by nanoparticles not supporting a surface plasmon resonance [25, 26]. In these reports, energy transfer quenching is attributed as the quenching mechanism, but given that no SPR is present (too small to support an SPR), then this is likely to be quenching by the non-free electron component of the dielectric function. In this work, we believe that low temperatures afford for a reduced "quenching" by the non-free electron component of the dielectric function, further facilitating enhanced fluorescence signatures.

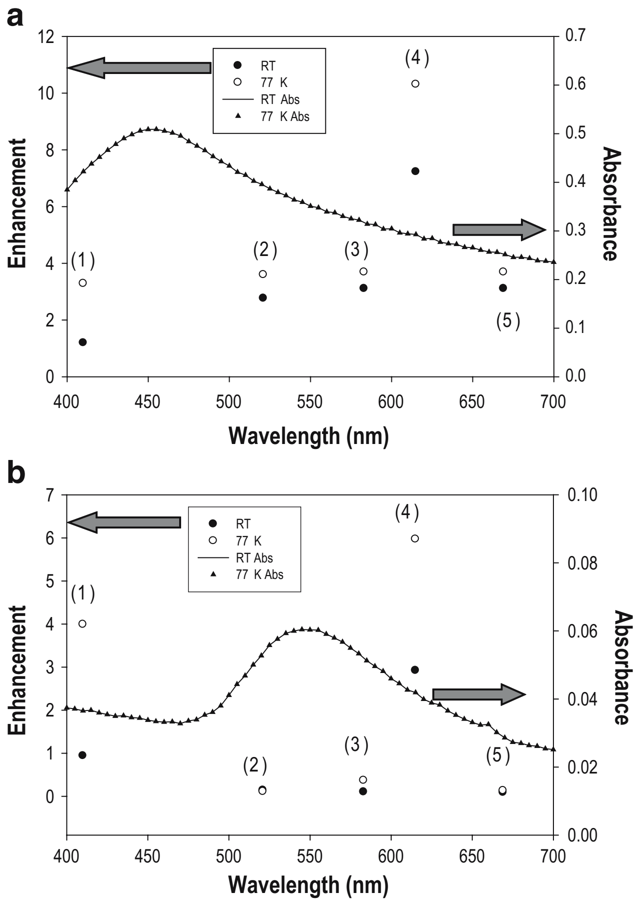


Fig. 3 Fluorescence enhancement factor vs different maximum emission wavelengths for various dyes on SiFs (**a**) and from 20 nm Au nanoparticles (**b**). Absorbance spectrum of SiFs (**a**) and 20 nm Au nanoparticles (**b**), at both RT and 77 K. *1* Acridine, *2* Acridine Orange, *3* Rhodamine B, *4* Cresyl Violet, *5* Nile Blue

In conclusion we report the further enhancement of MEF at low temperature, as compared to control samples. By comparing the fluorescence emission spectra of fluorophores from both glass and silver, any changes in excitation conditions for the two systems can be dismissed, as can effects due to line narrowing and traditional low temperature fluorophore fluorescence effects. Given that no changes in plasmon resonance were experimentally observed at low temperature, then we speculate that at low temperatures, a reduced "dampening" or reduced "quenching" of the fluorophore facilitates MEF through changes in the non-free electron component of the effective dielectric function. In this regard, it is widely known that the dielectric function of gold and silver is dependent on both the size and the environment of the metal [21]. Finally, we have studied a range of fluorophores over the visible region with both gold and silver nanoparticles. The enhancement factors are observed to be similar for both metals, across the wavelengths (400–660 nm), suggesting that the changes in dielectric function of both metals are similar at liquid nitrogen temperatures. These observations are not only helpful in understanding low temperature plasmon effects, but in our laboratories continued efforts to develop a unified plasmon-fluorophore theory.

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