Metal-enhanced fluorescence from copper substrates

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In this letter, the authors report the observation of metal-enhanced fluorescence emission from fluorophores deposited on copper (Cu) substrates. Different thicknesses of Cu particulate films (from 1 to 5 nm) were deposited onto glass slides using thermal vapor deposition. Fluorophores positioned in close proximity to the Cu films show fluorescence enhancement as a function of the Cu thickness increases, reaching a maximum (2.5-fold) at ≈3 nm. The findings strongly suggest that surface plasmons from Cu can radiate and therefore enhance a fluorophore's spectral properties, similar to observations reported for both silver and gold nanoparticle deposited substrates. © *2007 American Institute of Physics*. [DOI: 10.1063/1.2732185]

Fluorescence detection is a useful tool in medical diagnostics and biotechnology. While fluorescence can be a very sensitive technique, 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, metallic nanostructures have been used 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 fluorescence (MEF),[1] radiative decay engineering,[2] and also surface enhanced fluorescence by us.[5]

In recent years, our laboratories have both discovered and demonstrated many applications of MEF.[6–8] These have included the increased detectability and photostability of fluorophores,[6] improved DNA[9] and protein detection,[10] ultrafast plasmonic assays,[11] microwave-triggered chemiluminescence phenomena,[12,13] metal-enhanced phosphorescence,[14] and the application of plasmon enhancement to singlet oxygen generation.[15] Our laboratory's current mechanistic interpretation of MEF has been explained by a radiating plasmon model, whereby nonradiative energy transfer occurs from excited distal fluorophores to surface plasmons in noncontinuous films [Fig. 1(b)], in essence, a fluorophore induced mirror dipole in the metal, the surface plasmons in turn efficiently radiate the emission of the coupling of fluorophores.[5] This explanation has been facilitated by our recent observation of surface plasmon coupled emission,[16] whereby fluorophores near to a continuous metallic film can directionally radiate fluorophore emission at a unique angle from the back of the film.[16] This interpretation has also led to the development of plasmon coupled and enhanced chemiluminescence[17] and phosphorescence,[14] which involve both chemically induced electronic and triplet excited states coupling to surface plasmons, respectively. Our current explanation of plasmon-lumophore interactions is subtly different than our early reports,[1,2] where we postulated that it was the fluorophore itself that radiated [Fig. 1(a)], its photophysical properties, thought to be modified by a resonance interaction with the close proximity to surface plasmons.[1,2]

In addition, nearly all previous MEF studies were exclusively focused on fluorescence emission from various silvered surfaces, such as silver islands,[6] silver colloids,[18] silver

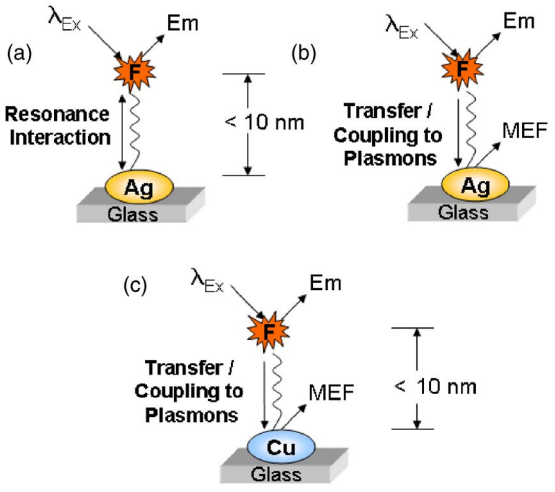

FIG. 1. (Color online) (a) Graphical representation of original interpretation of metal-enhanced fluorescence (MEF), (b) current interpretation for MEF on silver, and (c) MEF from copper. F: fluorophore, Ag: silver nanoparticles, and Cu: copper nanoparticles deposited using thermal vapor deposition.

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nanotriangles,[19] silver nanorods,[20] and fractal-like silvered surfaces.[21] When the fluorophores are in close proximity to silver particles, a significant number of experiments have shown that fluorescence emission intensities can be increased from sixfold to 3000-fold.[7] Recently, the observation of MEF from gold substrates has also been reported by our group.[22] Given the cost of generating both gold and silvered surfaces, it is thought that those substrates are not likely to find widespread MEF applications.

Further, the absorption cross section depends on the imaginary component of the metal, it is more than twice as large for copper (5.80) as those for silver (1.66) and gold (1.86) at approximately 520 nm (maximum emission of acridine orange). It is therefore expected that close-proximity fluorescence would be mostly quenched by the copper nanoparticles. On the other hand, copper also has a scattering component, which is thought responsible for the MEF effect.[5] Hence, MEF from copper nanoparticles is expected, but predicted to be less pronounced as compared to silver and gold, furthering our basic knowledge of MEF to develop a unified plasmon-fluorophore theory.

Subsequently, in this letter, we have investigated whether Cu, a relatively inexpensive metal as compared to gold and silver, can also be used as a substrate for MEF. Interestingly, we have observed an enhanced fluorescence emission (which is ≈2.5-fold brighter) from acridine orange near Cu nanoparticles as compared to a glass control substrate containing no nanoparticles. Furthermore, we have observed a shorter acridine orange fluorescence lifetime in close proximity to Cu nanostructures, which is in complete agreement with other reports and trends for metal-enhanced fluorescence from silver,[6] which describes the coupled system lifetime.

In this study, different thicknesses of Cu films were deposited with a deposition rate of 0.1 Å/s using thermal vapor deposition (model 306, Edwards). 300 μl of acridine orange ($1.0\times10^{-4}M$) in ethanol was sandwiched between both the glass slides (silane-prep slides) and the copper slides, respectively, as shown in Fig. 2(a), right insert. Fluorescence spectra were collected at an angle of 90° to the surface. Excitation light was incident to the bottom of the glass surface (evanescent wave excitation), as shown in Fig. 2(b), insert. Atomic force microscope (AFM) images were performed on a Molecular Imaging Picoplus microscope. Samples were imaged at a scan rate of 1 Hz with 512×512 pixel resolution in a tapping mode.

Figure 2(a) shows the fluorescence emission spectra of acridine orange on a 3 nm Cu film and on glass. It can be seen that acridine orange shows an enhanced fluorescence intensity (≈2.5-fold) on the Cu film when compared to the emission from glass (a control sample), where both spectra are identical when normalized [Fig. 2(b)]. Similar to the results for silver nanoparticles,[6] this indicates a mirrored fluorophore surface dipole on the copper nanoparticles. The real-color photograph inserts [Fig. 2(a), left insert] also provide additional visual evidence for enhanced emission from the Cu film. In this regard, it should be noted that the true metal-enhanced fluorescence enhancement factor (I_{silver}/I_{glass}) is actually ≈25-fold. This is because the MEF phenomenon is distance dependent, where evanescent wave excitation only penetrates ≈200 nm into solution,[23] and the MEF enhancement originates from only the first 20 nm of solution, therefore the observed 2.5-fold enhancement originates from only 10% of the sample volume, c.f. Fig. 2(a), right insert.

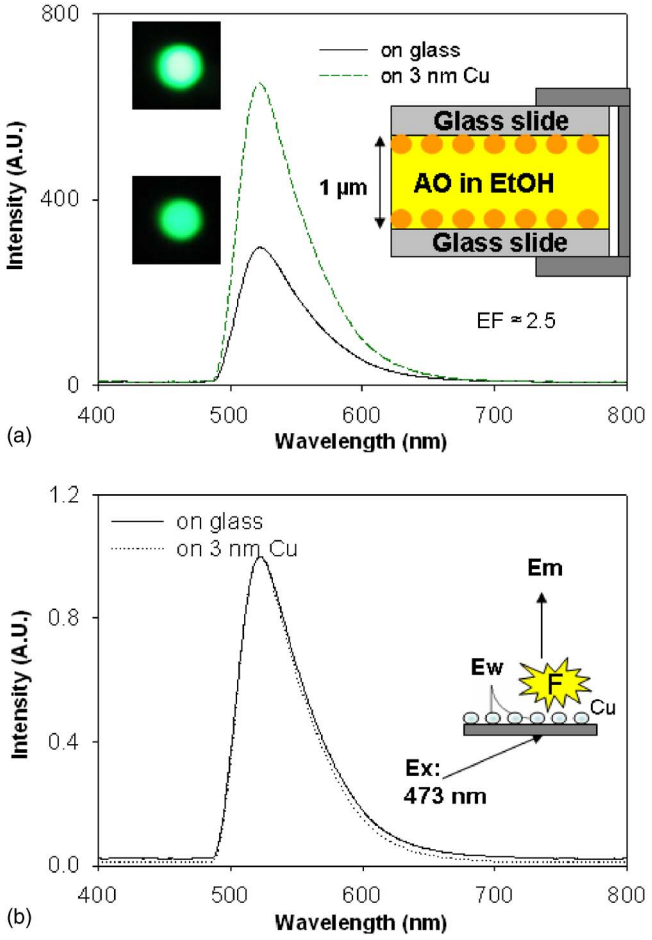

FIG. 2. (Color online) (a) Fluorescence emission spectra, λ_{ex}=473 nm, of acridine orange sandwiched between two coppered and uncoppered slides at room temperature [(a), left insert] and real-color photographs taken through an emission filter (488 nm). [(a), right insert] Schematic representation of the sample geometry. (b) The normalized emission spectra from both glass and copper. [(b), insert] Experimental setup. AO: acridine orange. Ew: evanescent wave. EF: enhancement factor.

The surface morphology of the Cu films with different thicknesses was characterized using AFM. AFM analysis of the Cu substrates revealed that the Cu was deposited as noncontinuous particulate films on glass for 1–3 nm (data not shown) after which continuous films (4 and 5 nm) were formed. This was also evident from the absorption spectra of the films (data not shown).

The fluorescence emission of acridine orange on the different thicknesses Cu films is summarized in Fig. 3(a). Because the plasmon scattering ability is low for Cu, 1/20th that of silver at 380 nm,[24] and due to the anticipated heterogeneity of the surface deposited Cu nanoparticles, we measured each sample 25 times and calculated the standard deviation for each [error bars shown in Fig. 3(a)]. It can be seen that the fluorescence is enhanced as the Cu thickness increases and reaches a maximum (2.5-fold) at 3 nm; however, the fluorescence is quenched when the Cu thickness was greater than 4 nm, which from both AFM analysis and absorption spectra revealed that continuous films were formed. This finding is consistent with trends observed for continuous and particulate silver[1] and gold films[22] and their influence on MEF.

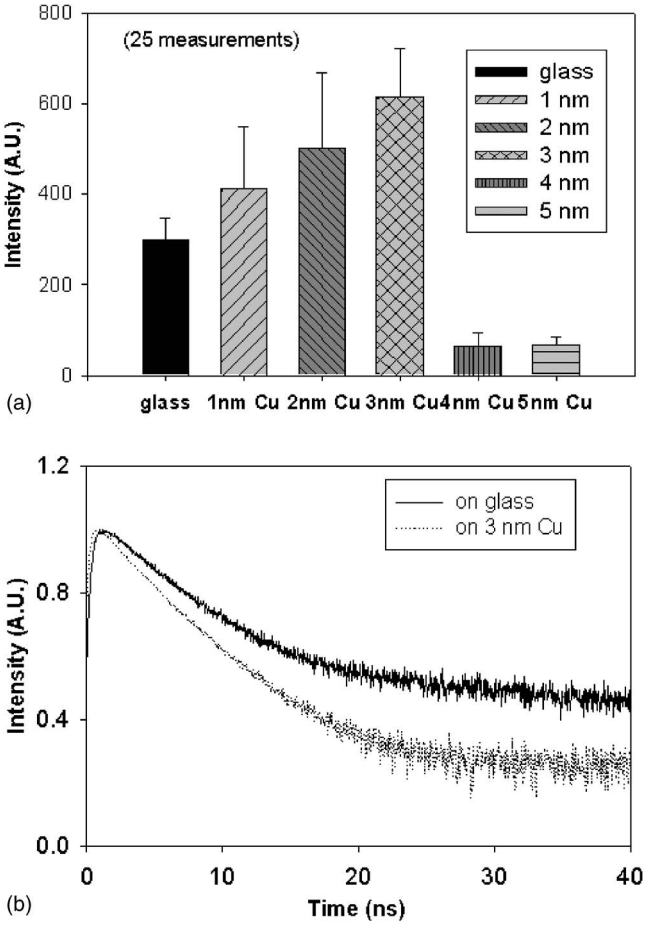

FIG. 3. (a) Average fluorescence emission intensity and standard deviation on different coppered/uncoppered substrates. (b) Fluorescence intensity decays of acridine orange from between coppered and uncoppered glass slides at room temperature.

We have also studied the intensity decays of acridine orange in close proximity to Cu, shown in Fig. 3(b). Using nonlinear least squares impulse reconvolution analysis, the respective lifetimes were calculated from those decays. We see both a reduced mean lifetime (τ_{mean} on Cu=3.4 ns) and amplitude-weighted lifetime ($\langle\tau\rangle$ on Cu=3.2 ns) for fluorophores near to Cu as compared to the glass control sample (τ_{mean} on glass=10 ns and $\langle\tau\rangle$ on glass=4.9 ns). These findings of reduced fluorophore lifetimes are consistent with our previously reported findings for fluorophores sandwiched between silver nanostructures, similarly suggesting that the radiating plasmon model[5,25] is a suitable description of the copper-fluorophore enhancement mechanism, Fig. 1(c). In this description, the lifetime of the fluorophore-metal system is reduced due to a faster and more efficient fluorophore-plasmon emission.

In conclusion, we report the observation of MEF from copper substrates. acridine orange in close proximity to copper nanoparticles can undergo enhanced fluorescence: a 2.5-fold increase was observed from 3 nm Cu films. This observation is helpful in our understanding not only for studying the interactions between plasmons and lumophores but also for potentially developing inexpensive substrates, which can be used in enhanced fluorescence based applications, such as DNA arrays and surface assays.

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