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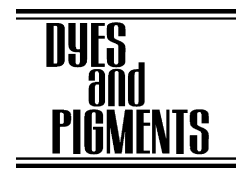


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Metal-enhanced fluorescence from paper substrates: Modified spectral properties of dyes for potential high-throughput surface analysis and assays and as an anti-counterfeiting technology

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Abstract

In this paper, we report the first observation of metal-enhanced fluorescence emission of fluorophores from paper substrates. The fluorescence intensity is ≈ 5 - and ≈ 9 -fold brighter from Rose Bengal (RB) and Pt(II) octaethylporphine (PtOEP), respectively, on silver colloid-deposited paper as compared to uncoated paper. In addition, a reduced lifetime of RB near to silver particles (0.77 ns) was observed as compared to the non-silvered control sample (0.86 ns). An equivalent photostability of RB on Ag colloid-deposited paper and uncoated paper was also observed. Our findings are potentially helpful in applications to high-throughput surface analysis and as the basis of an anti-counterfeiting technology. © 2007 Elsevier Ltd. All rights reserved.

Keywords: Fluorescence; Metal-enhanced fluorescence; Radiative decay engineering; Surface enhanced fluorescence; Plasmon controlled fluorescence; Radiating plasmons; Plasmon enhanced fluorescence; Plasmon enhanced luminescence

1. Introduction

Throughout the world, counterfeiters have forged virtually every kind of paper document or certificate, including checks, banknotes, passports, stock and bond certificates, automobile titles, bus and rail passes, even food stamps. “What one can make, another can copy” is an aphorism that widely supports the whole of the anti-counterfeiting industry [1]. In order to defeat the counterfeiters, paper products should be protected

by an advanced suite of security printing techniques. Recently, several products and technologies against counterfeiting and forgery such as Security Inks, Security Paper and AuthenticGuard™ Verifier have been introduced. In many of these anti-counterfeiting technologies, fluorescence plays a very important role, wherein the detected optical signal is used to identify the source information after being irradiated. Despite the benefits of using fluorescence, there is still a need to increase the detection limits because of the well-known photo-physical constraints of fluorophores, such as their typically low quantum yields, and generally poor photostability [2].

In the past 5 years, there have been significant developments using metallic nanoparticles to increase fluorescence signatures. Our group has subsequently named this technology as metal-enhanced fluorescence (MEF) [3–5]. Numerous experiments have shown that fluorescence emission intensities can be increased from up to 3000-fold [6], when fluorophores are in close proximity to silver nanoparticles. Subsequently,

Abbreviations: MEF, metal-enhanced fluorescence; MEP, metal-enhanced phosphorescence; RB, Rose Bengal; PtOEP, Pt(II) octaethylporphine; SiFs, silver island films; IRF, instrument response function.

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fluorescence emission has been studied from various silvered surfaces, such as silver islands [7], silver colloids [8], silver nano-triangles [9], silver nano-rods [10] and even fractal-like silvered surfaces [11]. Recently, our group has also reported the observation of MEF from gold colloid coated substrates [12]. However, previously our studies were exclusively focused on depositing metal nanoparticles on *rigid surfaces*, such as glass and polymer substrates. However, paper substrates are also likely to find widespread MEF applications, such as in paper based assays [13] or in anti-counterfeiting applications. In addition, due to metal-chromophore distant-dependent interaction, modified system lifetimes, photostabilities, angular-dependent emissions, and enhanced intensities have been reported [14–16]. Subsequently, these systems become promising candidates for anti-counterfeiting applications.

Our laboratory *postulated* that it was the fluorophore itself that radiated in metal-enhanced fluorescence, Fig. 1 – top, its photophysical properties were thought to be modified by a resonance interaction with the close proximity to surface plasmons. Due to surface plasmon effects, whereby fluorophores near to a *continuous metallic film* can directionally radiate fluorophore emission at a unique angle from the back of the film [16], our current explanation of plasmon–lumophore interactions is *subtly different* from that of our earlier reports [4]. Our current mechanistic interpretation of MEF has been explained by a model whereby non-radiative energy transfer occurs from excited distal fluorophores to surface plasmons in non-continuous films (Fig. 1 – bottom). The surface plasmons in turn, efficiently radiate the emission of the coupling fluorophores. This interpretation has also led to the development of plasmon coupled and enhanced chemiluminescence [17] and phosphorescence [18,19], which involve both chemically induced electronic and triplet excited states coupling to surface plasmons, respectively. These observations have also led us to postulate the first unified plasmon–fluorophore theory [20].

In this paper, we report our MEF findings from paper substrates using both Rose Bengal (RB) and Pt(II) octaethylporphyrine (PtOEP). The fluorescence intensity is ≈ 5 - and ≈ 9 -fold brighter from Rose Bengal (RB) and Pt(II) octaethylporphyrine (PtOEP), respectively, on Ag colloid-deposited paper as compared to uncoated paper. In addition, lifetime and photostability measurements of the fluorophores on silvered paper have also been studied. Our observations are potentially helpful in applications to high-throughput surface analysis and as the basis of an anti-counterfeiting technology.

2. Experimental

Rose Bengal, PtOEP, silver nitrate, trisodium citrate, and ethanol (HPLC/spectrophotometric grade) were obtained from Sigma–Aldrich. All chemicals were used as received. Filter paper (pore size 0.2 μm , Grade No. 43) was obtained from Filtrapor.

The synthesis of silver colloids was performed using the following procedure as described previously: 2 ml of 1.16 mM trisodium citrate solution was added drop wise to

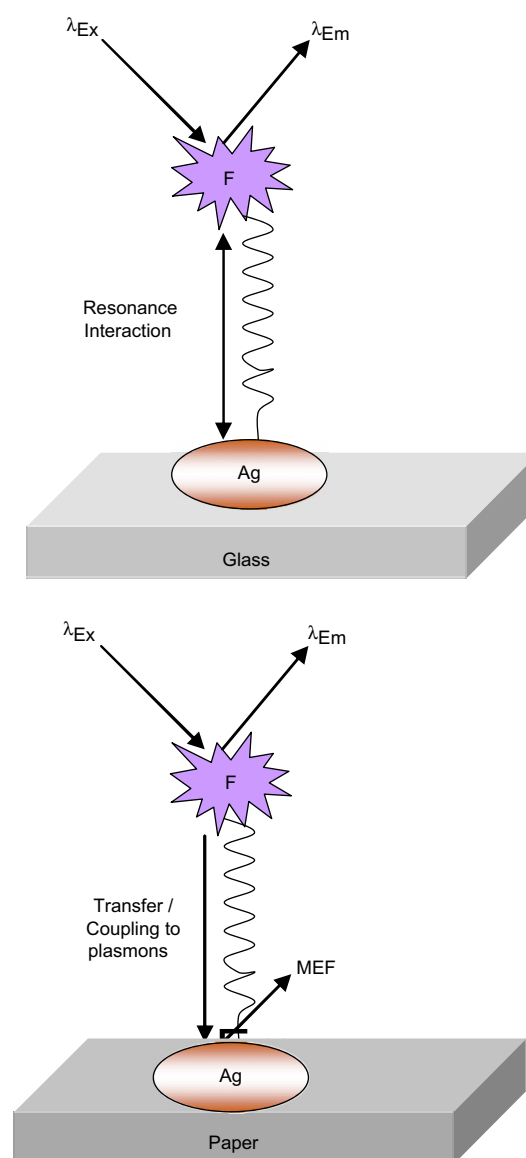


Fig. 1. Graphical representation of original interpretation of Metal-Enhanced Fluorescence (MEF) on silvered glass (top), current interpretation for Metal-Enhanced Fluorescence on silvered paper substrate (bottom). F – Fluorophore, MEF – Metal-Enhanced Fluorescence, Ag – silver colloids. Silver colloids are embedded in paper and deposited onto glass.

a heated (90 °C) 98 ml aqueous solution of 0.65 mM silver nitrate while stirring. The mixture was kept heated for 10 min, and then cooled in ice until use. This procedure yields ca. 50 nm silver colloids as confirmed by TEM analysis [21,22].

A drop of silver colloid solution was pipetted onto filter paper and the aqueous solution was allowed to evaporate. Rose Bengal (1.0×10^{-4} M) or PtOEP (1.0×10^{-4} M) of 20 μl in ethanol solution was subsequently pipetted onto the Ag colloid dried paper.

Fluorescence collection measurements were performed at 45° to the excitation, through a long pass filter, using a Fiber Optic Spectrometer (HD2000) from Ocean Optics, Inc. The real-color photographs on Ag colloid-deposited paper and unsilvered paper were taken with an Olympus digital camera

(C-740, 3.2 Mega Pixel, 10× optical zoom) using the same long pass filter that was used for the emission spectra.

Fluorescence lifetimes were measured using the Time-Correlated Single Photon Counting (TCSPC) technique, a PicoQuant modular fluorescence lifetime spectrometer (Fluo Time 100) with a PicoQuant 497 nm LED (PLS-8-2-1015) as the excitation light source. The intensity decays were analyzed in terms of the multi-exponential model:

$$I(t) = \sum_i \alpha_i \exp(-t/\tau_i) \quad (1)$$

where α_i is the amplitude and τ_i is the decay time, $\sum_i \alpha_i = 1.0$. The fractional contribution of each component to the steady state intensity is given by:

$$f_i = \frac{\alpha_i \tau_i}{\sum_j \alpha_j \tau_j} \quad (2)$$

The mean lifetime of the excited state is given by:

$$\bar{\tau} = \sum_i f_i \tau_i \quad (3)$$

and the amplitude weighted lifetime is given by:

$$\langle \tau \rangle = \sum_i \alpha_i \tau_i \quad (4)$$

The values of α_i and τ_i were determined by a nonlinear least squares impulse reconvolution analysis with a goodness-of-fit X^2 criterion.

3. Results and discussion

Fig. 2 – top and bottom shows the fluorescence emission spectra, $\lambda_{\text{ex}} = 532$ nm, for Rose Bengal and PtOEP dried onto paper and Ag colloid-deposited paper, respectively. We observed that the enhanced fluorescence intensity was >5-fold and >9-fold brighter for RB and PtOEP, respectively, from Ag colloid-deposited paper as compared to unsilvered paper (a control sample) where both spectra are identical when normalized (not shown). The real-color photograph insets also provide additional visual evidence for the enhanced emission from the fluorophores on the paper substrates. Interestingly, the background emission from the paper is broad and structureless when excited at 532 nm, but can be readily overcome with the use of luminescent probes and silver nanoparticles. We observed MEF from paper where the filter paper has a pore size of $\approx 0.5 \mu\text{m}$. Since a general print paper has a porous continuous texture, we speculate that we can also add Ag colloid (20–80 nm) and observe MEF from these substrates as well.

From Table 1 one can see that the amplitude weighted lifetime (τ) for Rose Bengal in a cuvette is shorter (0.75 ns) as compared to RB deposited on the filter paper (0.86 ns). This result is not unexpected and simply reflects the reduced mobility of the fluorophore on the paper [14]. However, on silvered

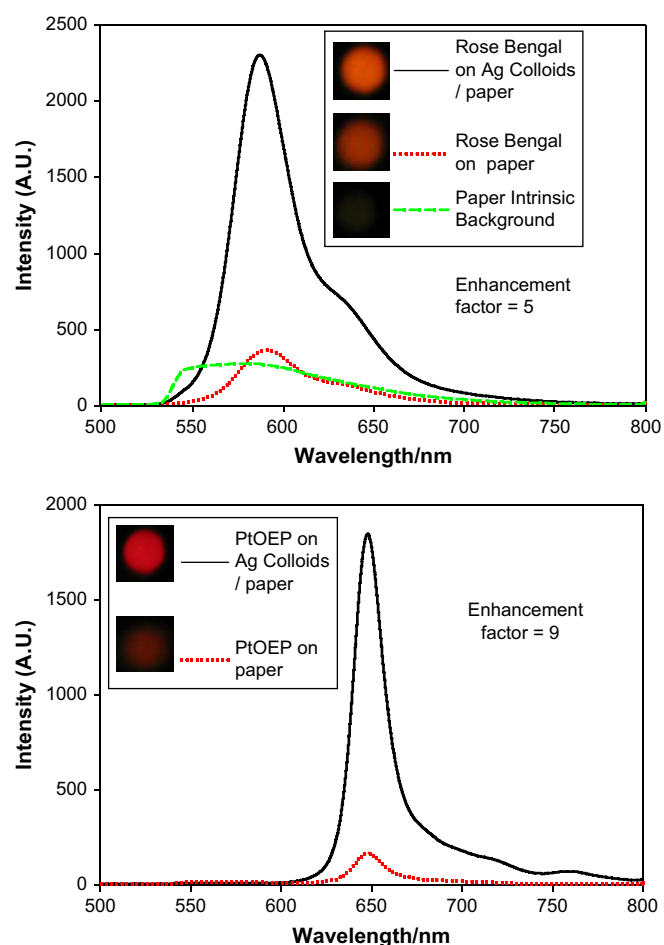


Fig. 2. Fluorescence emission spectra of Rose Bengal on paper and Ag colloid-deposited paper (top) and PtOEP on paper and Ag colloid-deposited paper (bottom). An excitation source of 532 nm was used for both samples.

paper, the amplitude weighted lifetime is shorter than on unsilvered paper, consistent with the numerous other reports on reduced fluorophore lifetimes for fluorophores near to silver by our group [7]. The fluorescence intensity decays can be seen in Fig. 3. These decays were used to calculate the respective lifetimes shown in Table 1 using nonlinear least squares impulse reconvolution analysis. From Fig. 3 and Table 1, we can see a reduced lifetime for Rose Bengal near to silver as compared to the control sample. This common observation for metal-enhanced fluorescence can be understood from the following set of equations for fluorophore lifetimes and quantum yields.

$$Q_0 = \frac{\Gamma}{\Gamma + k_{\text{nr}}} \quad (5)$$

$$\tau_0 = \frac{1}{\Gamma + k_{\text{nr}}} \quad (6)$$

where, Γ is the radiative decay rate, k_{nr} is the non-radiative decay rate, Q_0 is the quantum yield and τ_0 is the fluorescence lifetime.

Table 1
Fluorescence intensity decay parameters

	τ_1 (ns)	α_1 (%)	τ_2 (ns)	α_2 (%)	τ_3 (ns)	α_3 (%)	$\langle\tau\rangle$ (ns)	$\bar{\tau}$ (ns)	x^2
Paper	9.95	6.22	1.41	93.8	—	—	1.94	4.13	0.92
RB in solution	0.50	35.2	0.87	64.7	9.3	0.05	0.75	0.67	0.88
RB on paper	13.6	0.73	2.29	10.8	0.58	88.5	0.86	2.67	1.03
RB on Ag colloid-deposited paper	1.02	0.87	2.03	12.9	0.49	86.3	0.77	2.12	1.05

— Mean lifetime, $\langle\tau\rangle$ amplitude-weighted lifetime, RB-Rose Bengal.

In MEF, we have found that the enhanced fluorescence signal (Quantum yields $-Q_m$) of fluorophores in close proximity (<10 nm) to metallic nanostructures could be well described by the following equations:

$$Q_m = (I + I_m)/(I + I_m + k_{nr}) \quad (7)$$

where I is the unmodified radiative decay rate, I_m is the metal-modified *system* radiative decay rate and k_{nr} is the system non-radiative rate. Similarly, the metal-modified lifetime, τ_m , of a fluorophore is decreased by an increased radiative decay rate:

$$\tau_m = 1/(I + I_m + k_{nr}) \quad (8)$$

These equations result in most unusual predictions for fluorophore–metal combinations. From Eqs. (7) and (8), we can see that as the value of the system I_m increases, the *system* quantum yield Q_m increases, while the lifetime, τ_m , decreases. This is contrary to most observations in fluorescence where the free-space quantum yield, Q_0 , and lifetime, τ_0 , nearly always change in unison, cf. Eqs. (5) and (6). From Fig. 1 – bottom, a highly efficient coupling to surface plasmons, where the plasmons efficiently and quickly radiate the coupled emission, results in a reduced fluorophore lifetime and enhanced observed emission intensity, cf. Eqs. (7) and (8).

Finally, we measured the photostability (fluorescence intensity as a function of time) of Rose Bengal and PtOEP on both unsilvered paper and Ag colloid-deposited paper (Fig. 4). Applying the same laser power we observed significantly more fluorescence (integrated area) from Ag colloid-deposited

paper. However, when we attenuate the laser power on the Ag colloid-deposited paper to match the same initial emission intensity as observed on unsilvered paper, we see little if any enhancement in photostability. This result is not so surprising when we consider that the amplitude weighted lifetime only changed from 0.88 to 0.77 ns on silvered paper as compared to unsilvered paper. This is because a fluorophore's photostability is partly governed by its radiative lifetime; molecules with shorter lifetimes being more photostable as there is less time for excited state photochemical reactions to occur, for example, photo-oxidation [2].

In addition, the use of the millisecond decay time PtOEP complex also allows us to overcome the intrinsic paper background, both in terms of emission intensity and also

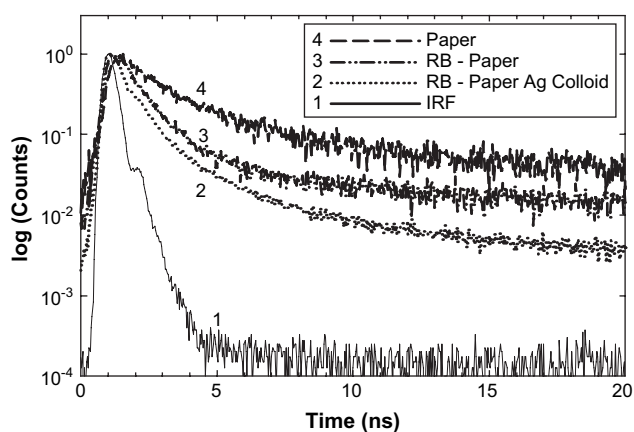


Fig. 3. Fluorescence intensity decays of Rose Bengal (RB) on paper and Ag colloid-deposited paper. IRF – Instrument Response Function.

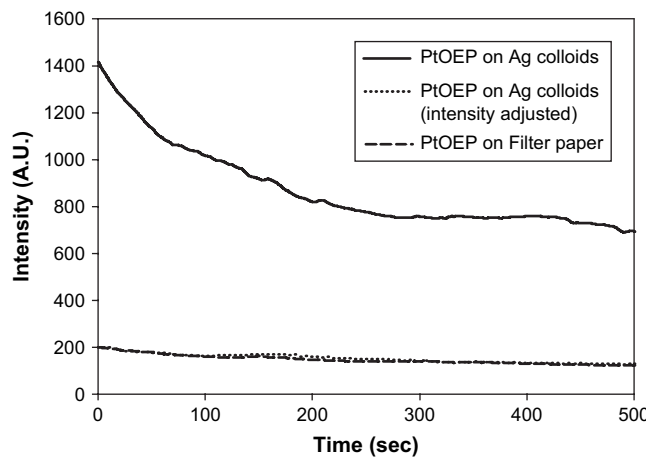
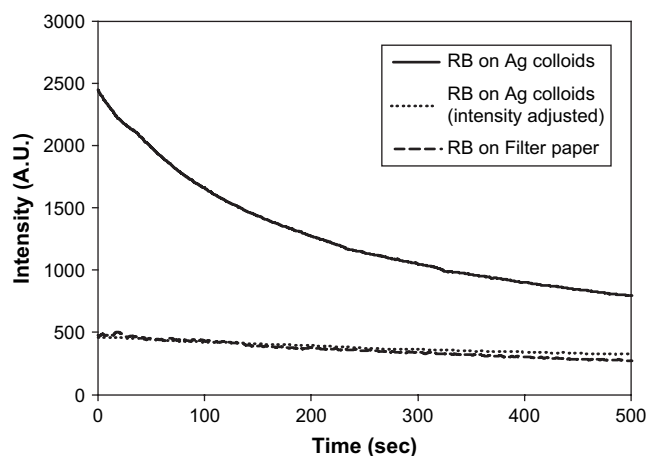


Fig. 4. Photostability of Rose Bengal (RB) on paper and Ag colloid-deposited paper (top) and PtOEP on paper and Ag colloid-deposited paper (bottom).

temporally, where the paper chosen was found to have an amplitude weighted lifetime around 1.94 ns. Therefore, it may be possible to use fluorescence/luminescence off-gating techniques [2] to overcome the fluorescence lifetime background. MEF from paper substrates can potentially be developed for high-throughput surface analysis. The MEF technology could well be used as an anti-counterfeiting measure, whereby dye/fluorophore's proximity to silver, affords for (a) enhanced luminescence signatures [6], (b) reduced lifetimes [6], (c) directional luminescence [12] and (d) enhanced photostabilities [6]. It is highly unlikely that these fluorophore–metal system properties can be counterfeited by generic printing/copying press methodologies.

4. Conclusions

In this paper, we have reported the first observation of metal-enhanced fluorescence from a paper substrate. The luminescence intensity of Rose Bengal and PtOEP is increased by 5- and 9-fold, respectively, on an Ag colloid-deposited paper substrate as compared to an identical control sample containing no silver. We also observed that Rose Bengal displayed a shorter fluorescence lifetime and an equivalent photostability on Ag deposited paper substrates. As we have shown, the background luminescence emission from standard filter paper can be quite large. MEF subsequently provides an opportunity to overcome this background emission. Given the significant advances in ink jet printing technology in the last 10 years [22], it is highly likely that silver nanoparticles can be printed onto paper substrates also [23]. Patterned paper could then find numerous applications in both anti-counterfeiting technologies and surface assay arrays. Work is currently underway in our laboratory in this regard.

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