J Fluoresc (2012) 22:1659–1662
DOI 10.1007/s10895-012-1132-3

ORIGINAL PAPER

Reduced Lifetimes are Directly Correlated with Excitation Irradiance in Metal-Enhanced Fluorescence (MEF)

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Received: 31 August 2012 / Accepted: 1 October 2012 / Published online: 9 October 2012

Abstract We describe a fundamental observation in Metal-Enhanced Fluorescence (MEF), which has become a leading technology in the life sciences today, namely, how the lifetime of fluorophores near-to metallic plasmon-supporting silver islands/nanoparticles, modulates as a function of excitation power irradiance. This finding is in stark contrast to that observed in classical far-field fluorescence spectroscopy, where excitation power does not influence fluorophore radiative decay/lifetime.

Keywords Metal-enhanced fluorescence · Plasmon-enhanced fluorescence · Surface enhanced fluorescence · Excitation volumetric effect · Near-field fluorescence

Introduction

In the last 150 years since Sir George Stokes discovered fluorescence [1] we have significantly increased our understanding of fluorescence by developing/discovering new fluorescence principles, new fluorophores, advanced instrumentation and forever more elegant approaches of data analysis [1]. One-hundred and fifty years later, fluorescence approaches and instrumentation are now both widely practiced and entrenched throughout the Life Sciences [1]. In all of these applications of fluorescence spectroscopy today, fluorescent molecules, (fluorophores), are for the most part considered to be emitting into a homogenous environment, free space, with little interaction or consideration for the complex interactions and emission properties of fluorophores less than one wavelength of light away from a substrate which can fundamentally alter both its absorption and emission properties, the so called near-field condition [2, 3].

In contrast to classical far-field fluorescence, we know very little as a community about the interactions of fluorophores in the near-field, near-field fluorescence. The discipline has however attracted much interest in the past 5 years or so [4, 5], where it has been realized that significantly enhanced emission intensities and reduced fluorophore lifetimes (enhanced fluorophore photostability) have particular utility, particularly in single molecule detection [6, 7], biochemical assays [8, 9] and in microscopy [10]. One particular near-field fluorescence discipline, originally termed Metal-Enhanced Fluorescence (MEF) by Geddes [2], has attracted significant attention [4].

Originally, electrodynamic descriptions of oscillating dipoles near-to metallic objects [4] have been used to describe fluorophore radiative rate modifications near-to metals. However, it has been shown that these treatments of the near-field fluorescence are indeed incorrect [4, 11], the nature of the enhanced near-field fluorescence being subtly more elegant and underpinned by a mechanism whereby fluorophores' non-radiatively couple to/induce surface plasmon oscillations in the near-field, the metallic particle surface plasmons themselves emitting the coupled quanta [3, 11]. While this difference in mechanism is subtle, it does have profound implications in fluorescence spectroscopy, some of which have started to be realized [11, 12]. In this paper we subsequently further extend the fundamentals of metal-enhanced fluorescence (MEF) and show how far-field excitation irradiance (i.e. laser power) can profoundly affect the near-field volume, Fig. 1, and therefore the mean or amplitude weighted fluorescence lifetime of a heterogeneous sample containing *both* far- and near-field positioned fluorophores. The significance of this observation cannot be overstated, where the excitation irradiance in classical fluorescence spectroscopy has little to no bearing on the observed fluorescence lifetimes [1].

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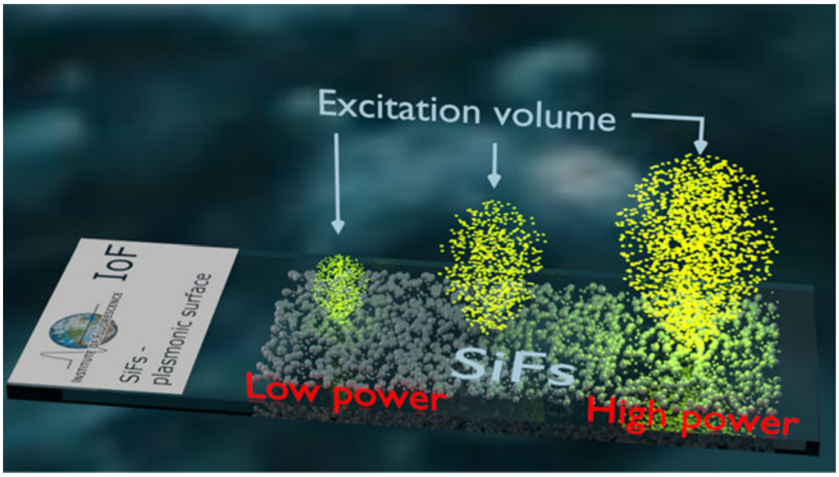

Fig. 1 Illustration of the MEF-EVE effect

Experimental

To demonstrate this new fundamental effect in near-field fluorescence spectroscopy, we have chosen to use a Ruthenium dye with a free space lifetime ≈800 ns, solution bound between Silver Island Films (SiFs). The SIFs were prepared as previously described [13]. Approximately 50 µL of a water solution of tris(2,2′-bipyridyl)dichlororuthenium(II) hexahydrate ($Ru(bpy)_3^{2-}$) was sandwiched between a clean glass slide and a silver island coated glass slide. The reference sample was recorded on otherwise identical sample of $Ru(bpy)_3^{2-}$ in a standard 1 cm quartz cell. Time-resolved fluorescence decays were recorded at different excitation power on a Horiba Jobin Yvon Tem-Pro fluorescence lifetime system employing the time-correlated single photon counting (TCSPC) technique, with a TBX-04 picosecond detection module. The excitation source was a pulsed NanoLED-470LH centered at 474 nm running at 250 KHz.

The time-resolved fluorescence intensity decays were fitted to a three exponential decay model in the software package DAS6.4 from Horiba,

$$I(t) = \sum_{i}^{k} \alpha_i \exp\left(-\frac{t}{\tau_i}\right) \tag{1}$$

where $k=3$ and α_i and τ_i are the pre-exponential factors $\left(\sum_{i=1}^{k} \alpha_i\right)$ and the decay times, respectively. The fractional contribution of each component to the steady-state intensity is given by

$$f_i = \frac{\alpha_i \tau_i}{\sum_{j=1}^{k} \alpha_j \tau_j} \tag{2}$$

The mean lifetime of the excited state is given by

$$\bar{\tau} = \sum_{i=1}^{k} f_i \tau_i \tag{3}$$

and the amplitude-weighted lifetime is given by

$$\langle \tau \rangle = \sum_{i=1}^{k} \alpha_i \tau_i \tag{4}$$

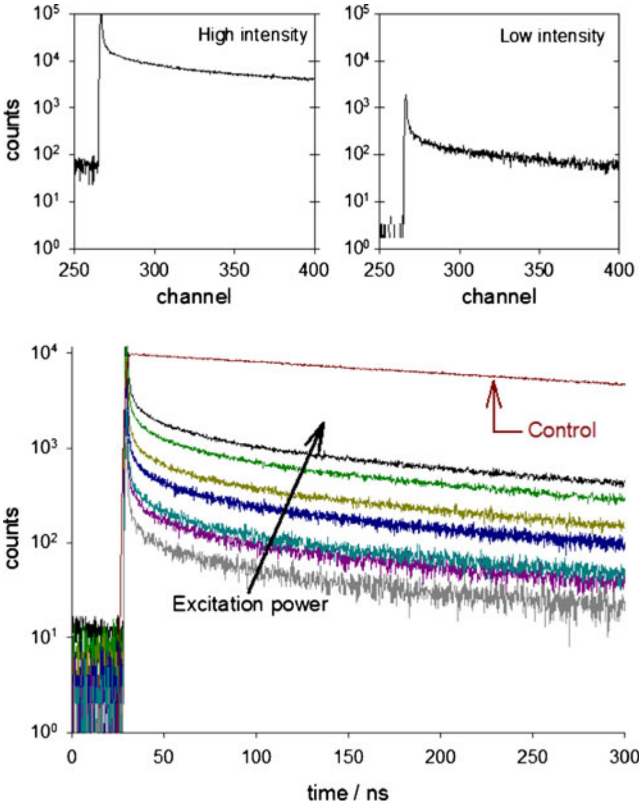

Fig. 2 The *lower panel* shows time-resolved fluorescence decay curves recorded for a range of different excitation powers of a Ruthenium dye on a SiF surface. The excitation wavelength was 474 nm and the emission was collected through a RazorEdge 488 nm filter combined with longpass filters centred at 550 nm and 590 nm. The *upper panel* shows decay curves recorded at an excitation power of 32.6 µW (high intensity) and at 0.03 µW low intensity

Table 1 Fitting results for $Ru \times (bpy)_3^{2-}$ sandwiched between SiFs

Power/μW	f_1/%	τ_1/ns	f_2/%	τ_2/ns	f_3/%	τ_3/ns	$\overline{\tau}/ns$	<τ>/ns	χ^2
32.60	99.9	1.05±0.02	0.03	24.2±0.47	0.11	231±1.47	1.30	1.05	1.60
23.08	99.9	0.98±0.02	0.03	21.1±0.53	0.09	203±1.65	1.18	0.99	1.36
12.98	99.9	0.97±0.03	0.03	22.2±0.55	0.09	223±2.03	1.18	0.98	1.29
10.31	99.9	0.88±0.03	0.02	22.1±0.60	0.08	218±2.32	1.07	0.88	1.19
8.19	99.9	0.99±0.04	0.03	22.0±0.60	0.09	220±2.33	1.19	0.99	1.16
3.26	99.9	1.01±0.06	0.03	22.8±1.29	0.09	228±3.88	1.22	1.01	1.11
1.03	99.9	1.31±0.07	0.05	24.4±1.14	0.16	225±3.66	1.69	1.31	1.15
0.03	94.9	2.15±0.18	1.17	32.3±3.17	3.90	261±11.3	12.6	2.26	1.12

$\overline{\tau}$ mean lifetime

<τ> Amplitude weighted lifetime

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

Results and Discussion

Figure 2 shows the fluorescence intensity decays of the Ruthenium dye sandwiched between the silver island plates. As the excitation power is increased, the decays show a greater amplitude shorter component, Fig. 2 top left and right, when the time window for data collection is fixed and both a high and low power set of decays are compared. As shown in Table 1, both the mean and amplitude weighted lifetimes decrease as a function of laser power irradiance on the SiFs surface, >10-fold for 0.03⟶32.60 μW, but remain virtually unchanged on a glass-glass control sample Table 2, which contains no silver nanoparticles and where the near-field MEF condition is not present [4]. Figure 2 also shows the significantly reduced lifetime of the Ruthenium dye near to SiFs as compared to that measured for the control sample. Remarkably, the amplitude of the short lived component (the plasmon coupled component) increases as the laser power increases. This finding is in stark contrast to what is traditionally observed in classical fluorescence spectroscopy [1], where laser excitation power has little if any effect on fluorescence lifetimes, i.e. on the glass-glass control sample.

While at first this finding might seem surprising, it can readily be explained by a change in the near-field excitation volume, which increases as a function of laser power, as shown in the cartoon depiction of Fig. 1, and theoretically described in our recent paper describing metal-enhanced Fluorescence excitation volumetric effects [12]. In our samples, as the laser power is increased, the enhanced near-field both excitation and coupling volume also increases [12] and so many more fluorophores couple-to/induce metallic surface plasmons and cause MEF. Given that the plasmon lifetime is very short, thought to be on the order of picoseconds [4] as compared to the near-microsecond decay time of the ruthenium dye, then the time-resolved intensity decays show very fast short-lived components, which increase in amplitude as a function of increasing laser power, consistent with Fig. 2 and Table 1.

Table 2 Fitting results for $Ru(bpy)_3^{2-}$ in a standard 1 cm quartz cell

Power/μW	f_1/%	τ_1/ns	f_2/%	τ_2/ns	$\overline{\tau}/ns$	<τ>/ns	χ^2
32.5	2.08	5.89 +/– 0.16	97.92	365.6 +/– 0.5	358.1	358.1	1.51
4.09			100	362.9 +/– 0.8	362.9	362.9	1.24
0.32			100	367.8 +/– 0.4	367.8	367.8	1.07

$\overline{\tau}$ mean lifetime

<τ> Amplitude weighted lifetime

To further support our hypothesis we have also calculated the steady-state fluorescence intensity as a function of laser power, by integrating the intensity decays. Figure 3 subsequently shows that the fluorescence is substantially increased as a function of excitation irradiance for the same sample,

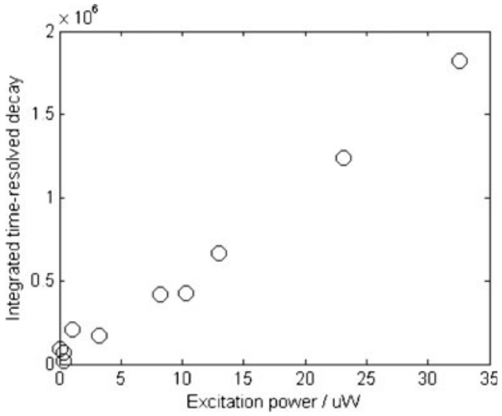

Fig. 3 Integrated time-resolved intensity decay (proportional to the steady-state intensity) as shown in Fig. 2 plotted as a function of excitation power

consistent with our recent MEF EVE postulate [12] and our observations here of reduced lifetimes as a function of increased laser power.

Conclusions

In conclusion, we have described a new fundamental observation in near-field fluorescence spectroscopy, whereby the lifetime of fluorophores in a heterogeneous system consisting of both near- and far-field fluorophores, is reduced as a function of increased lased power. This finding is likely to find utility in microscopy and in fluorescence-based immunoassays, where absolute brightness (detectability) coupled with a much improved photostability (reduced lifetime) are primary concerns [7].

Acknowledgment The authors acknowledge the support of the NIH NIAID 2 U54 AI057168-09. Salary support from the Institute of Fluorescence and UMBC is also acknowledged.

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