

Metal-Enhanced Fluorescence from Chromium Nanodeposits

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Over the last several years, our laboratory has described in detail the favorable effects of fluorophores in close proximity to metallic nanoparticles. Metal nanoparticles comprised of gold, silver, copper, and zinc show enhanced fluorescence intensities and photostabilities for fluorophores positioned within 10 nm of the particles. In this paper we show that, in addition to these metals, chromium nanodeposits can also enhance the fluorescence of close proximity fluorophores, with probes such as fluorescein showing a greater than 8-fold enhancement. However, in contrast to gold, silver, and copper nanoparticles, fluorophores in close-proximity to chromium nanodeposits do not display reduced radiative lifetimes, suggesting that an enhanced electric field component (enhanced excitation rate) is the dominate mode of emission enhancement, similar to recent work by our laboratory for zinc nanoparticles. In addition, we also show the metal-sandwich geometries used in previous metal-enhanced fluorescence studies can give erroneous enhanced fluorescence readings, if care is not taken to normalize the excitation intensity/irradiance.

Introduction

Metal-enhanced fluorescence (MEF) is a technique where the fluorescence emission of many fluorophores with different metals has been shown to be enhanced via fluorophore-metal nanoparticle interactions.^{1–5} MEF is thought to be comprised of two mechanisms (1) an electric field effect and (2) an induced plasmon effect. The mechanism of enhancement for the electric field effect is based on an increase of the fluorophores absorption cross-section when in close proximity (<10 nm) to a metal nanoparticle that is exposed to an electric field.⁶ In this mechanism excitation and subsequent emission of the fluorophore are increased, while the fluorescence lifetime is not affected. The second mechanism, the induced plasmon effect, is thought to be based on the partial coupling of excited states of the fluorophore and surface plasmons on the metal nanoparticle (coupled quanta).⁷ The induced plasmon effect is deduced by (1) an increase in the fluorescence emission of the fluorophore-metal nanoparticle unified system, where the spectral characteristics of the fluorophore remain unchanged, and (2) a decrease in the fluorescence lifetime.

The fundamental nature of MEF makes it applicable to a vast spectrum of scientific and technological fields.¹ Thus it is desirable to explore the effects of different metals on the characteristics of the fluorophores for these applications. To date several metals have been used for MEF including silver,⁸ gold,⁹ copper,¹⁰ aluminum,¹¹ and most recently zinc.¹² Another metal, chromium, is routinely used as an undercoat for the deposition of gold¹³ or silver thin films due to its adhesive nature, though its potential usefulness in MEF has not hitherto been explored. In this paper we subsequently show that chromium nanodeposits can also be used as a substrate for MEF applications. Chromium nanodeposits of various thicknesses were deposited onto glass microscope slides, which were characterized by optical absorption and atomic force microscopy (AFM) techniques. Several

fluorophores with emission wavelengths ranging from 520–610 nm were deposited onto chromium substrates in a sandwich sample format. Enhancement of fluorescence emission from fluorophores only with a high free-space quantum yield was observed. Furthermore, no notable change in the fluorophore's radiative lifetime was observed, which strongly suggests that the electric field effect is the dominant mechanism in MEF from chromium nanodeposits.

Experimental Section

Materials. All fluorophores including fluorescein, fluorescein isothiocyanate (FITC), Acridine Orange, Rose Bengal, Rhodamine B, Sulforhodamine 101 (S101), and Rhodamine 101 (used as a standard in lifetime measurements) and silane-prep glass microscope slides were purchased from Sigma-Aldrich Chemical company (Milwaukee, WI). Chromium nanostructured films of various thicknesses were deposited onto silane-prep glass microscope slides by AccuCoat, Inc. Rochester, NY.

Preparation of Sandwich Format Samples for Metal-Enhanced Fluorescence Measurements. A solution of 200 μL of a fluorophore (500 μM) was sandwiched between two glass slides for the control and between one glass and one chromium nanostructured film, or in the case of Figures 5 and 6, between two chromium nanostructured films. Each dye was excited with a source of appropriate wavelength and the fluorescence emission spectra and real-color photographs collected as described below.

Optical Spectroscopy and Real-Color Photographs. The absorption spectra of the chromium nanostructured films of varying thicknesses were collected using a Varian Cary 50 UV–vis spectrophotometer. Fluorescence spectra of the fluorophores were measured with blank glass sandwiches and glass-nanostructured film sandwiches using an Ocean Optics HD2000 fluorometer. A Canon Powershot S50 digital camera was used for real-color photographs of each of the dyes. The spectra were plotted and analyzed with SigmaPlot software. Frequency domain lifetime measurements of selected fluorophores were

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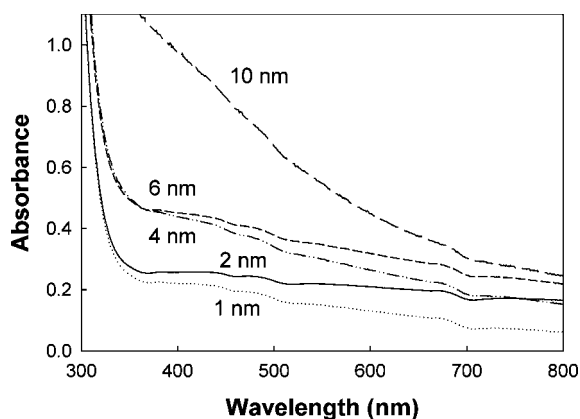


Figure 1. Absorption spectrum of metallic chromium for various thicknesses deposited onto glass microscope slides. These absorbance measurements were made with dry samples (in air). Glass was used as the blank control sample.

sequentially made in cuvettes, sandwiched in glass slides and in glass-nanostructured film sandwiches using a Horiba Jobin Yvon Multifrequency Fluorometer (MF²) with a 408 nm laser excitation source.

Frequency Domain and Phase Modulation Lifetime Measurements. Frequency domain lifetime measurements of Fluorescein and Sulforhodamine 101 (S101) were measured in cuvettes, glass slide sandwiches, and glass-chromium substrate sandwiches in a front-face geometry using a Horiba Jobin Yvon Multifrequency Fluorometer (MF²) with a 408 nm NanoLED pulsed laser-diode and a 495 nm long-pass emission filter. A cuvette of Rhodamine 101 in water was used as a standard reference with a reported lifetime of 4.32 ns.⁹ The phase and modulation data were fitted using a fitting program provided by Horiba Jobin Yvon.

Atomic Force Microscopy (AFM). 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 contact mode.

Mie Scattering Calculations. Mie scattering calculations were made for chromium nanoparticles in water using freeware MieCalc v1.5 software.

Results and Discussion

It is well-known that continuous and noncontinuous metal nanostructured films exhibit very different properties in metal-enhanced fluorescence.⁹ Therefore, it is important to assess the continuity for the various deposition thicknesses. This was accomplished by employing optical absorption spectroscopy and AFM. Figure 1 shows the absorption spectra of 1, 2, 4, 6, and 10 nm thick chromium nanodeposits, which are measured with the micro quartz balance in the thermal metal evaporator, used in this investigation. These thicknesses were selected to investigate the effect of thickness of chromium on the fluorescence emission. Chromium nanodeposits show an absorbance peak around 390 nm that is increased as the sample thickness is increased, and a broad absorption spectrum, which is indicative of the aggregation of the nanodeposits on the surface, a result of the thermal deposition. To visually confirm this hypothesis and to determine the morphology of the surface, images were obtained for the 2, 6, and 10 nm films by AFM and are shown in Figure 2. For a 2 nm chromium sample, several aggregates of chromium nanodeposits are clearly seen on the surface. It is interesting to note that little or no nanodeposits were present in between the larger aggregated deposits in this sample. Figure 2, panels B and C, shows that as more chromium nanodeposits are deposited onto the surface the gaps between the aggregates are occupied by the additional nanodeposits. This,

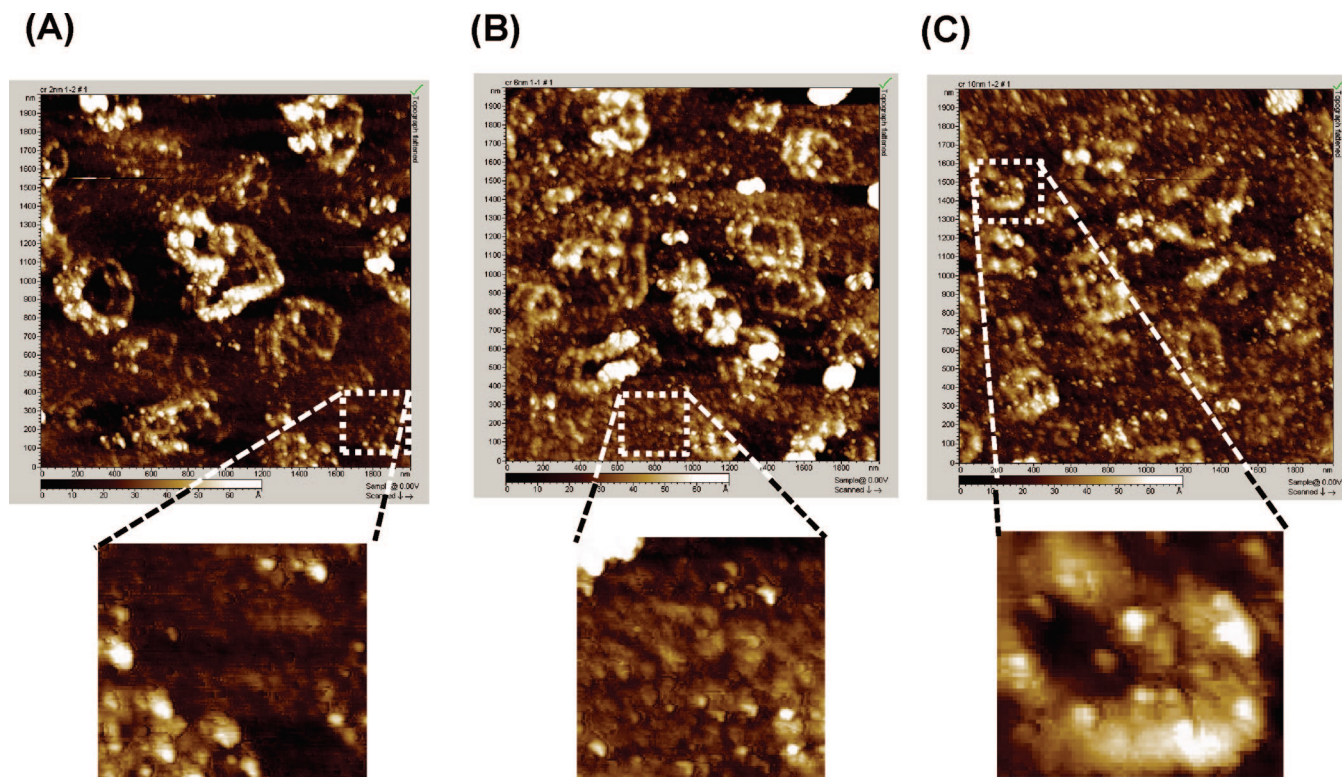


Figure 2. Atomic Force Microscope images of chromium nanostructured films with thicknesses of (A) 2, (B) 6, and (C) 10 nm using the contact-mode operation of the AFM. The thicknesses of the chromium nanostructured films were measured by the micro quartz balance in the thermal evaporator.

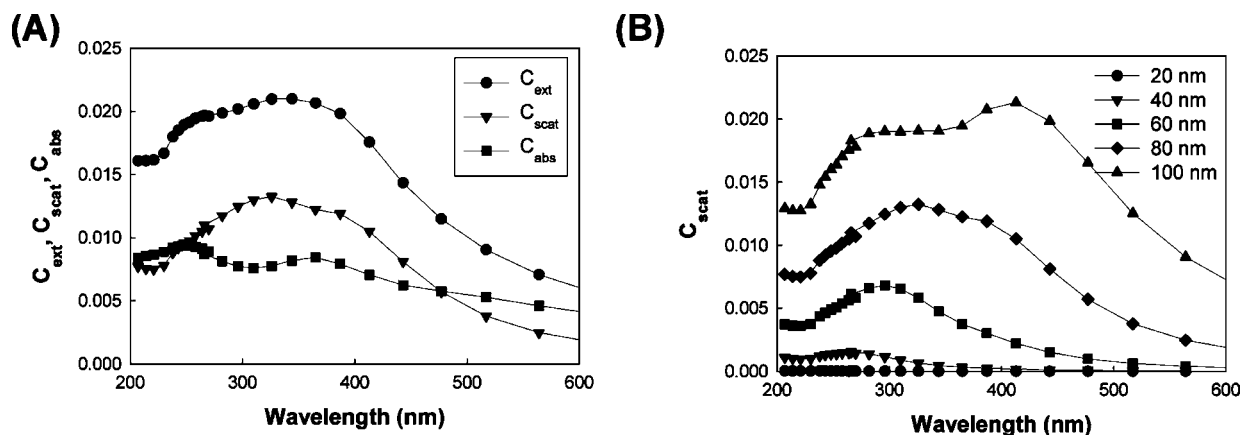


Figure 3. (A) Calculated Mie extinction, scattering and absorption cross section of a 80 nm diameter chromium nanoparticle. (B) Calculated Mie scattering cross section of various chromium nanoparticle diameters.

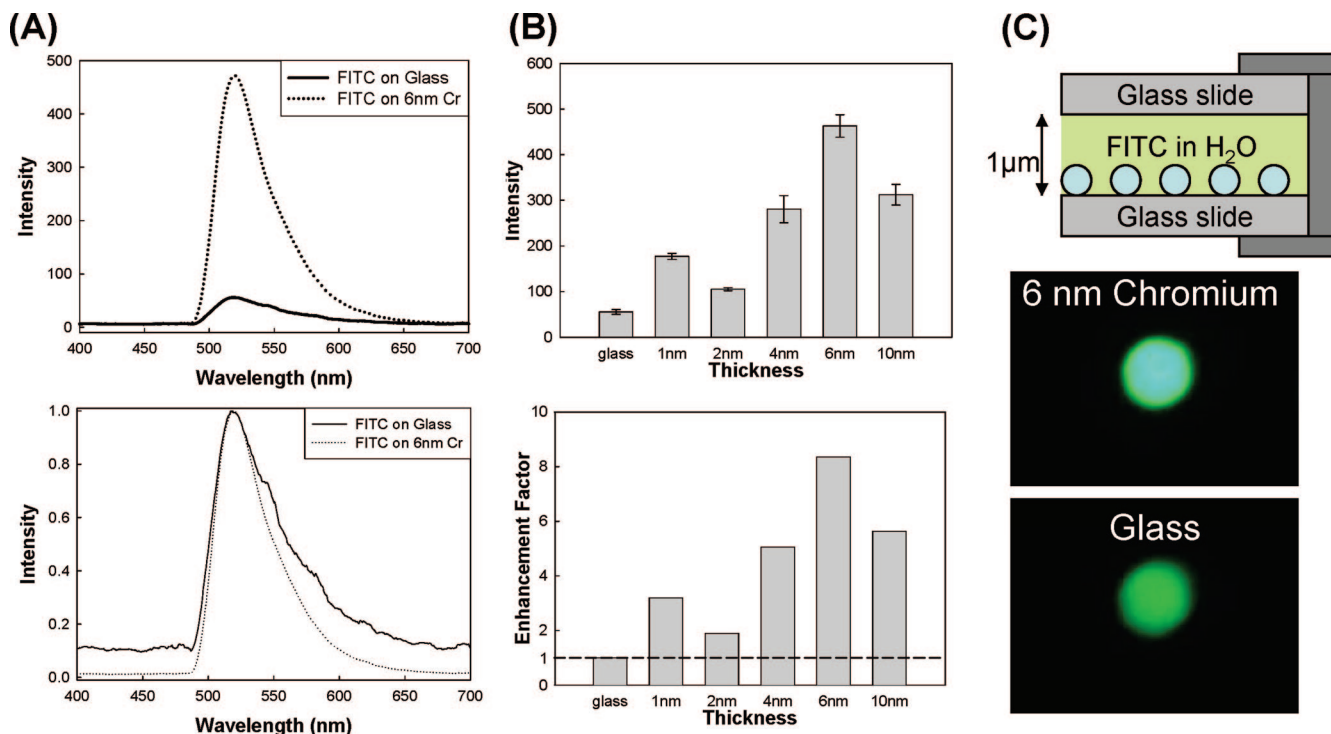


Figure 4. (A) Raw (top) and normalized (bottom) fluorescence emission spectrum of FITC from chromium (6 nm thick) and glass substrates. (B) Fluorescence emission intensity of FITC measured (at 540 nm) from glass and chromium substrates (top) and calculated fluorescence enhancement factor for FITC. Average of 5 measurements are shown. (C) Schematic of the chromium sample geometry (top) and real-color photographs of FITC emission from 6 nm chromium (center) and glass (bottom) substrates, taken through an emission filter.

in essence, accounts for the broadening of the absorption spectra, i.e., Figure 1.

Mie calculations are a very useful tool to predict the size- and wavelength-dependent absorption and scattering efficiencies of metal nanoparticles in different dielectric medium surrounding the nanoparticles. These calculations also provide preliminary indications of the possible mechanism of the MEF phenomenon from metal nanostructures of interest. In this regard, we performed Mie calculations for chromium nanoparticles of various diameters in water, c.f. Figure 3. Figure 3A shows the extinction cross-section for an 80 nm chromium nanoparticle (size guesstimated from AFM analysis), which is comprised of two components: absorption and a scattering cross-section. It is calculated in Figure 3A that the scattering component dominates the absorption component of the extinction spectrum across the 200–600 nm wavelength range. Furthermore, it is predicted through these calculations that the scattering compo-

nent increases and broadens to longer wavelengths as a function of particle size (Figure 3B). Interestingly, the plasmon effect accounts for the second enhancement effect in MEF, which becomes significant for particles greater than 80 nm in diameter.

Figure 4A shows the raw and normalized emission spectra obtained from FITC on glass and chromium substrates. From these measurements it is clear that MEF is in fact observed, and that enhancement increases as a function of thickness (Figure 4B) for the film thicknesses up to 6 nm. It should be noted that fluorescence enhancement in the case of the 10 nm thickness is less than that of the 6 nm, which further suggests that the film is continuous at this thickness, thus agreeing with the absorption measurements and AFM images shown in Figures 1 and 2, respectively. The normalized fluorophore emission spectra in Figure 4A (bottom) show that the spectral characteristics are preserved in chromium-based MEF. Finally, Figure 4C (top) shows the experimental geometry implemented in these

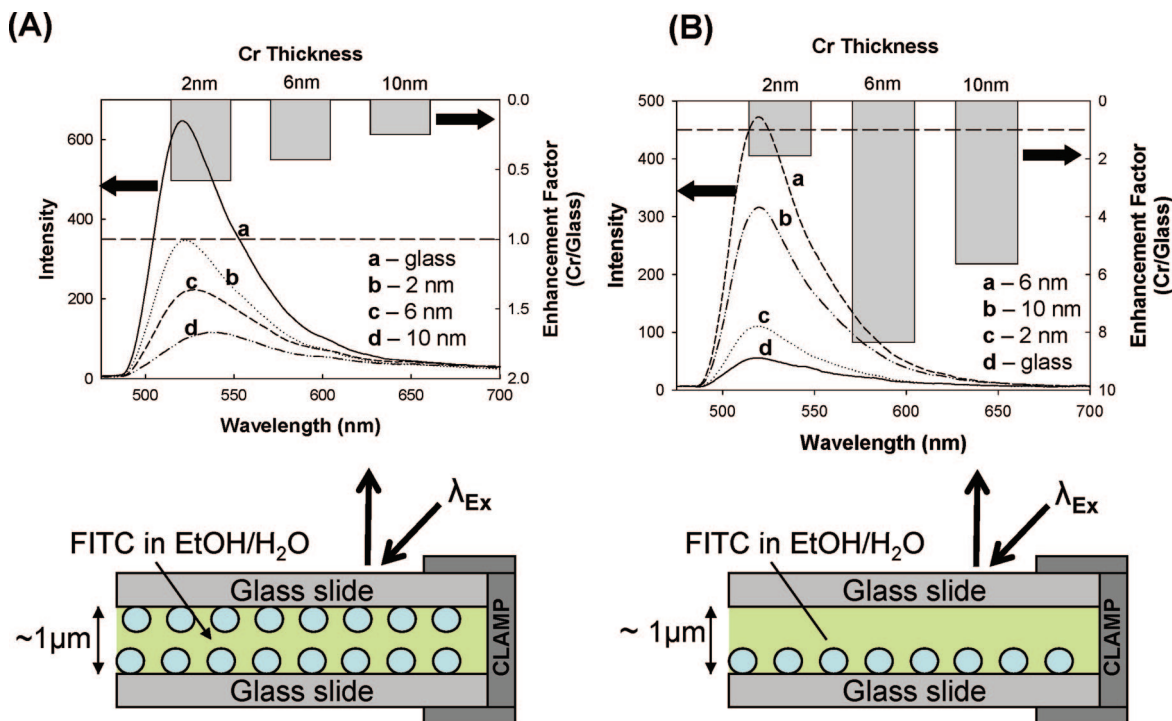


Figure 5. (A) Emission spectra and geometry schematic of FITC sandwiched between two chromium-deposited glass slides of varying thicknesses and (B) FITC sandwiched between one blank glass slide and one chromium-deposited glass slide.

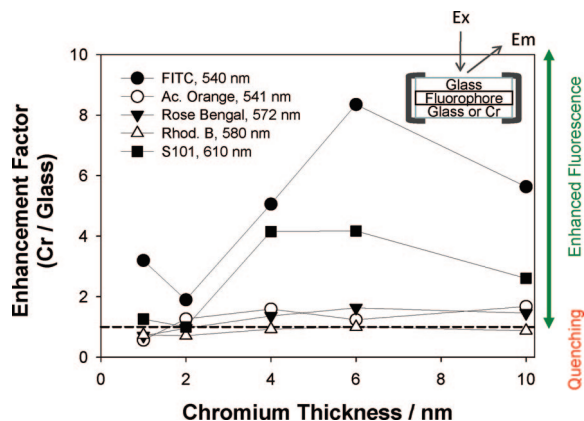


Figure 6. Calculated fluorescence enhancement factor for various fluorophores deposited onto chromium substrates. The enhancement factor is calculated as the ratio of the intensity at the wavelengths indicated on different chromium substrates as compared to glass (a control sample).

measurements, and the real-color photographs (bottom) taken through an emission filter, which depicts the enhanced fluorescence as seen by the digital camera and by eye.

It should be noted that the geometry depicted in Figure 4C (top) was designed after we observed that the conventional metal–metal sandwich format¹⁴ demonstrated a decrease in fluorescence emission as a function of film thickness, shown in Figure 5A. We predicted that this trend was due to an increase in back-reflection of excitation light (off of the top slide containing the chromium nanodeposits) as a function of increasing thickness. This back-reflection is believed to decrease the amount of excitation light available to the fluorophores in the sandwiched solution. Figure 5B (bottom) depicts the geometry that was designed in order to measure the true MEF of the chromium nanostructured films, as well as test this back-reflection hypothesis. Here, the top half of the sandwich is a plane glass slide, thus eliminating the backscatter and effectively

illuminating the control and sample with equal intensity. Figure 5B (top) shows the enhancement factors obtained with this new geometry for three thicknesses, where the enhancement for all 3 film thicknesses is greater than 1.

While the experimental geometry shown in Figure 5B (bottom) addresses the issue of decreased excitation of fluorophores due to back-reflected light from the top glass slide, it is also important to comment on the effect of back-reflection of light from the bottom slide containing the chromium nanodeposits on the observed enhanced fluorescence. In this experimental configuration, one can expect two factors influencing the fluorescence emission: (1) back-scattered excitation and (2) back-scattered fluorescence emission. While the former is eliminated with the use of emission filters, the latter is expected to contribute to the increased fluorescence emission from chromium nanodeposits, an additional advantage of these substrates, to an extent that was not determined here. The back-scattered fluorescence emission is also expected to result in longer fluorescein lifetimes due to the increase in path length for which the emission travels.

As previously stated several fluorophores were measured on the chromium substrates for MEF and are given in the Supporting Information S1–S4. The summary of these results are shown in Figure 6, where we see a general enhancement in fluorescence intensity for FITC, Rose Bengal, S101, and Acridine Orange, though Rhodamine B is quenched. The fluorophores with the highest quantum yields (Supporting Information, Table S1), namely FITC and S101 exhibit the greatest enhancement factors thus suggesting that the electric field effect is the dominant mechanism in chromium MEF.¹⁵ It should be noted that in contrast, the induced plasmon enhancement mechanism, results in the enhancement factor being proportional to $1/Q_0$, where Q_0 is the free space quantum yield. It is also important to note that the electric field around metal nanodeposits varies with the wavelength and thus the enhancement factor for fluorophores absorbing light at different wavelengths will also vary with wavelength. In addition, the

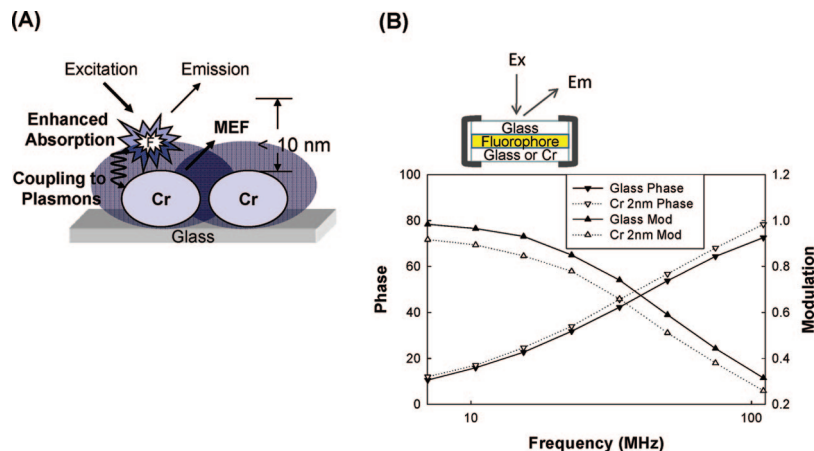


Figure 7. (A) Experimental geometry (top), frequency-domain phase, and modulation of fluorescein (in deionized water) in a cuvette and sandwiched between 2 nm thick chromium substrates. The experimental geometry shows the orientation of samples and the placement of fluorophores with respect to the samples. (B) Schematic representation of MEF phenomena.

TABLE 1: Lifetime Values for Fluorescein and Sulforhodamine 101 (S101) Measured from Glass and Chromium Deposited Substrates

fluorophore	substrate	τ (ns)	χ^2
fluorescein	cuvette	4.33	1.10
	glass	4.54	0.98
	2 nm Cr	5.01	1.08
	6 nm Cr	5.33	1.10
S101	cuvette	4.51	1.15
	glass	4.10	1.06
	2 nm Cr	4.53	1.14

efficiency of coupling of fluorescence emission to surface plasmons of the metal also varies with wavelength. It is expected that the enhancement of fluorescence emission from fluorophores with red-shifted emission spectra is larger. The observation that Rhodamine B (QY = 0.7) and S101 (QY = 0.9) exhibited different degrees of enhancement (Figure 6) can be explained by the differences in the extent of electric fields and the efficiency of fluorescence emission coupled to surface plasmons, i.e., both enhancement mechanisms are wavelength dependent phenomena.

An additional observation (Supporting Information S5) displays the extinction components of a 200 nm diameter particle overlaid with the normalized emission spectra of the various fluorescent molecules. This data would suggest that there should be no real variation in enhancement between the various fluorophores if the induced-plasmon effect was the dominant mechanism (Figure 7A).⁶ What we see, however, is that this relationship is not observed, suggesting that the induced-plasmon coupling effect is negligible.⁶ Furthermore, we observed no notable decrease in the fluorescence lifetime for the fluorophores when in the presence of metal nanostructures (Figure 7 and Table 1), which again strongly suggests that there is little to no enhancement from the induced-plasmon effect (mirror dipole effect). Finally, we observed that no shift in the absorption and emission peak for FITC on chromium nanodeposits when compared to on glass slides.

Conclusions

In this paper we have demonstrated the effectiveness of chromium as a suitable metal for MEF. Several fluorophores and excitation wavelengths were used and a general trend of enhancement of fluorescence was observed. The extent of enhancement factor was found to be larger for fluorophores with a high free-space quantum yield as compared to those with low

quantum yield, suggesting that the electric-field effect is the dominating mechanism in MEF from chromium nanodeposits. Moreover, the absence of a second, much shorter fluorescence lifetime when in the presence of chromium nanostructures also suggests that there is no notable induced-plasmon effect. In contrast, a reduced lifetime coupled with enhanced intensities has been observed for Ag,⁸ Al,¹¹ and Cu¹⁰ nanodeposits. A recent report for Zn nanostructures¹² also shows a similar trend to that observed for Cr here. Our results show that chromium can be used as an inexpensive alternative metal to other commonly used metals in MEF applications.

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Supporting Information Available: Additional figures and a table of experimental data. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Supporting Information for the Manuscript “Metal-Enhanced Fluorescence from Chromium Nanodeposits”, Pribik *et al.*

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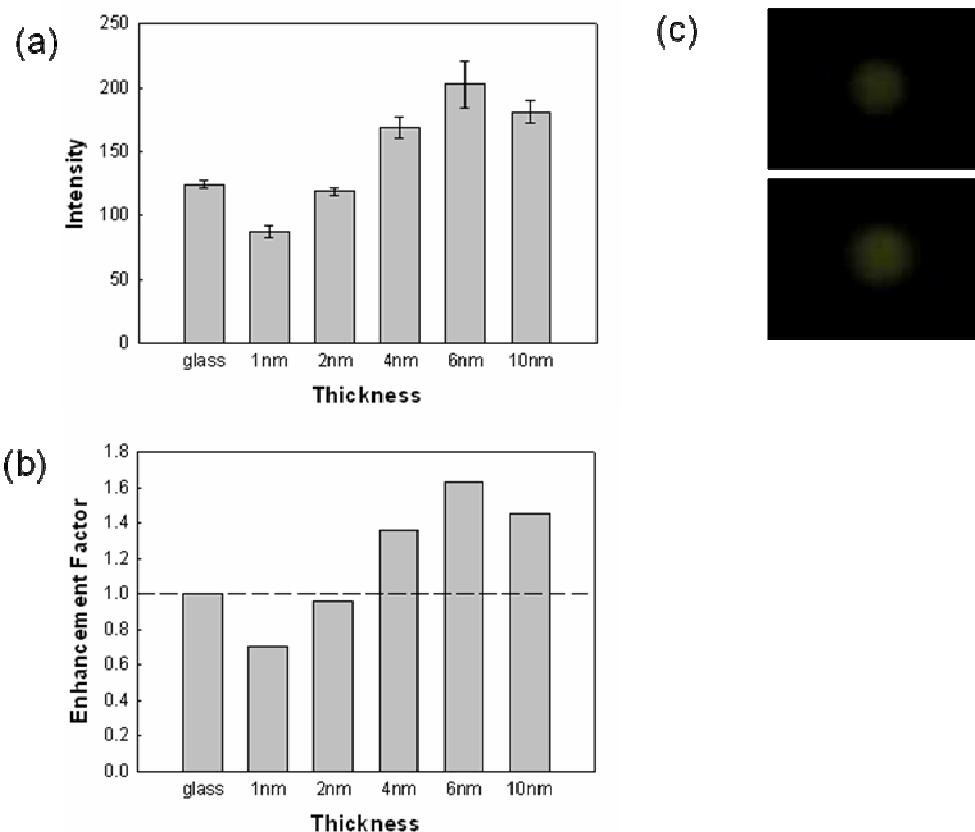
Supporting Information: Table S1: Summary of metal-enhanced fluorescence measurements for FITC, Acridine Orange, Rose Bengal, Rhodamine B, and Sulforhodamine 101 on chromium-deposited slides and their corresponding free-space quantum yield obtained from the literature.

Fluorophore	$\lambda_{EM} \text{ max (nm)}$	Q _o	Enhancement Factor Range	[Ref]
FITC	540*	0.92	1.90 - 8.35	1
Acridine Orange	541	0.20	0.56 - 1.68	2
Rose Bengal	572	0.11	0.70 - 1.63	3
Rhodamine B	580	0.70**	0.71 - 1.00	4
S101	610	0.90***	1.00 - 4.17	1

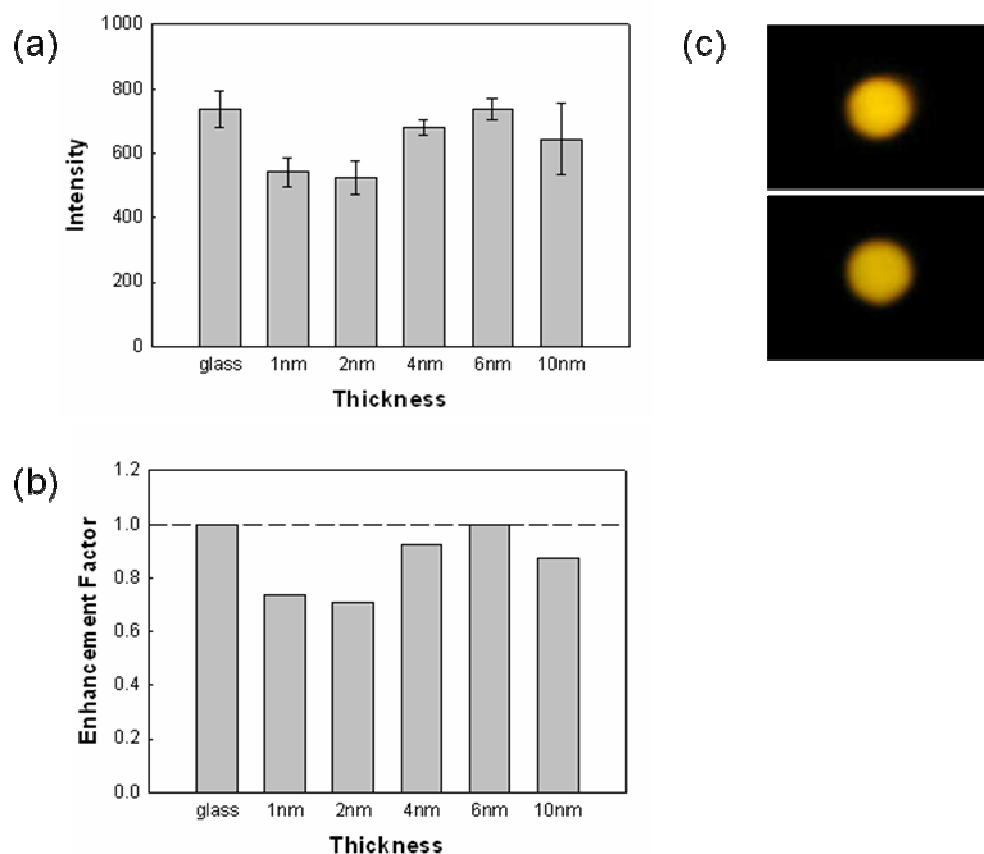
* in EtOH/H₂O

** in Borate Buffer

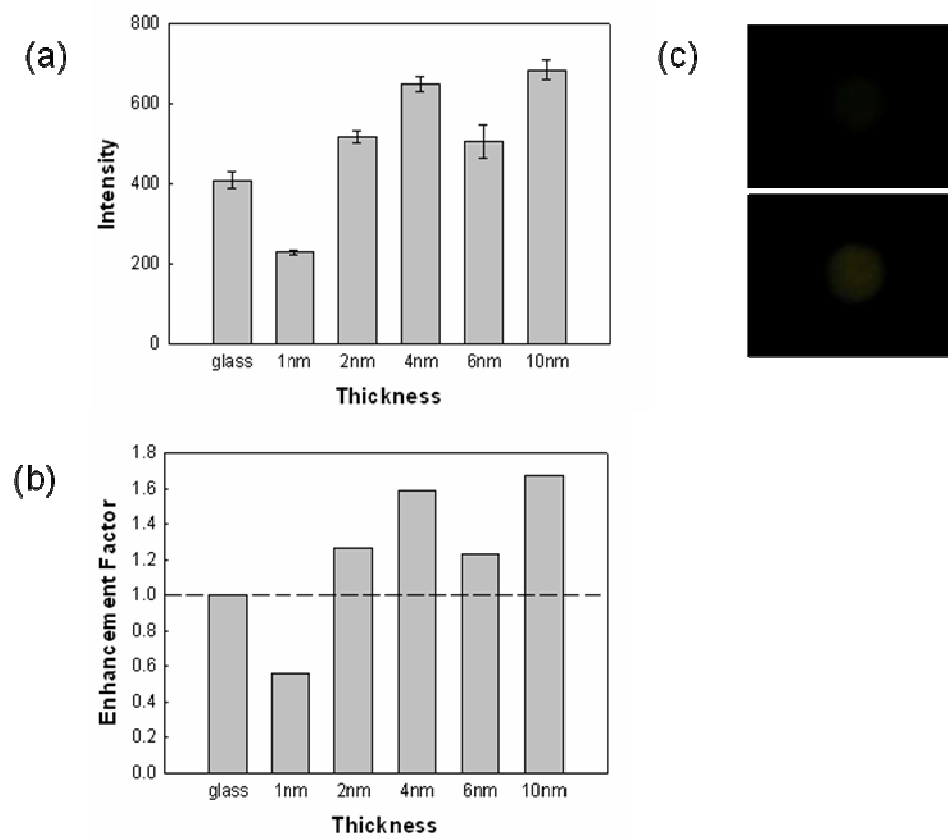
*** in EtOH



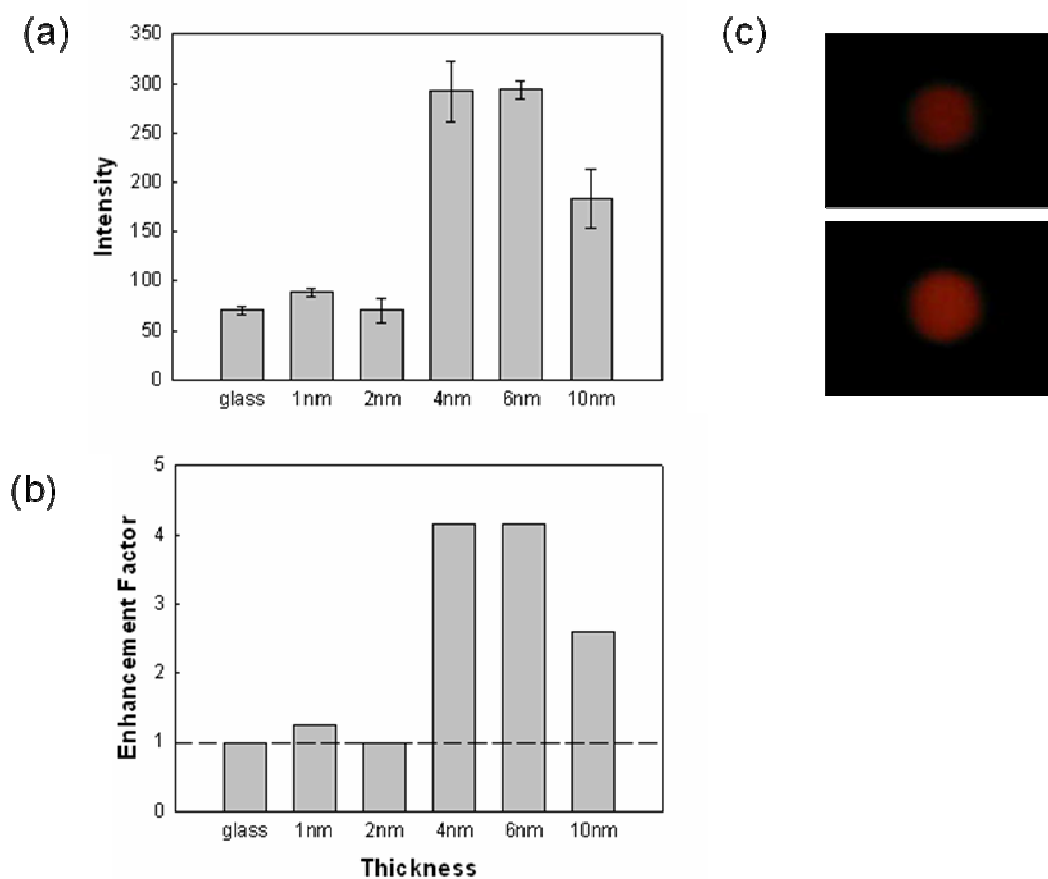
Supporting Information: Figure S1. Metal-enhanced fluorescence of Rose Bengal from chromium-deposited slides of varying thicknesses (a) and the corresponding enhancement factor (b). Real color photographs of Rose Bengal on glass (top) and from 6 nm chromium (bottom) with an emission filter placed in front of the camera lens (c).



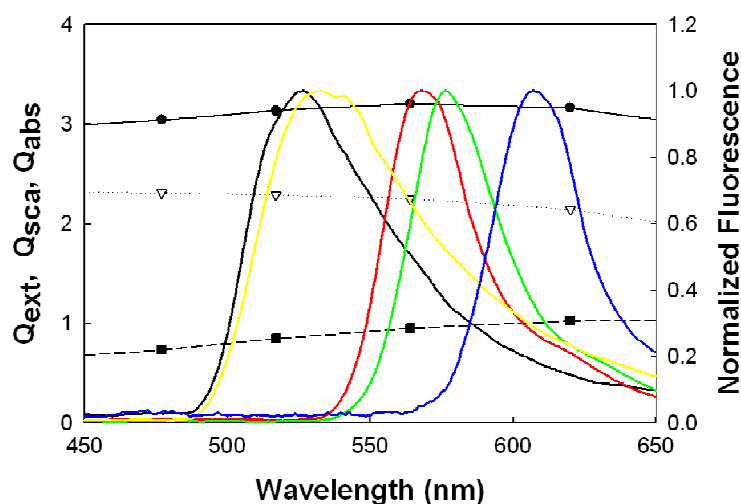
Supporting Information: Figure S2. Metal-enhanced fluorescence of Rhodamine B from chromium-deposited slides of varying thicknesses (a) and the corresponding enhancement/quenching factor (b). Real color photographs of Rhodamine B on glass (top) and from 6 nm chromium (bottom) with an emission filter placed in front of the camera lens (c).



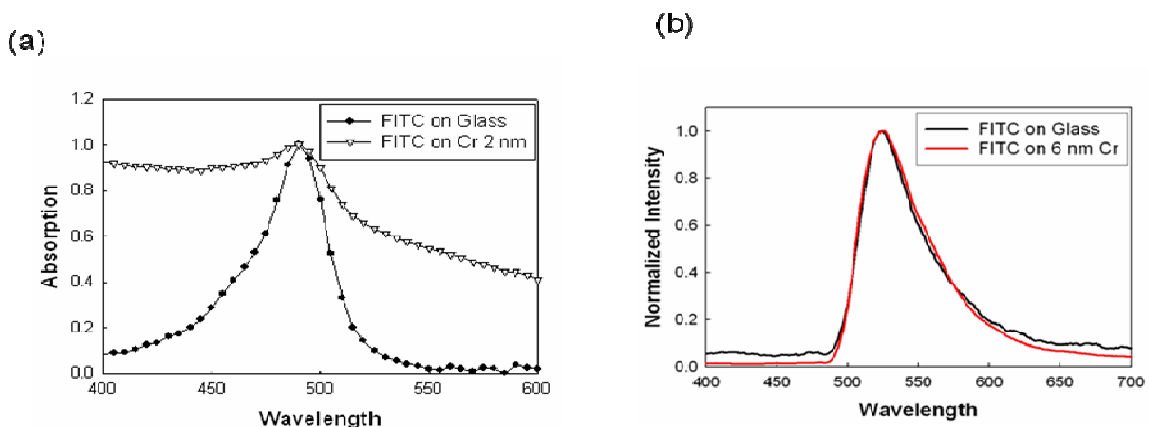
Supporting Information: Figure S3. Metal-enhanced fluorescence of Acridine Orange from chromium-deposited slides of varying thicknesses (a) and the corresponding enhancement/quenching factor (b). Real color photographs of Acridine Orange on glass (top) and from 10 nm chromium (bottom) with an emission filter placed in front of the camera lens (c).



Supporting Information: Figure S4. Metal-enhanced fluorescence of Sulforhodamine 101 (S101) from chromium-deposited slides of varying thicknesses (a) and the corresponding enhancement/quenching factor (b). Real color photographs of Sulforhodamine B on glass (top) and from 6 nm chromium (bottom) with an emission filter placed in front of the camera lens (c).



Supporting Information: Figure S5. Calculated Mie extinction, scattering and absorption cross section for 200nm diameter chromium nanoparticles and the emission spectra of the fluorophores used in this study. The lack of any discrete trends in enhancement factor vs. emission spectra overlap with the scattering portion of the nanoparticle extinction, suggest no induced plasmon effect is present, and indeed the enhanced intensities are due to an electric-field enhancement only.



Supporting Information: Figure S6. (A) Excitation and **(B)** emission spectrum of FITC on glass and 2 nm Cr.

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