

# Metal-Enhanced Fluorescence from Gold Surfaces: Angular Dependent Emission

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**Abstract** The first observation of Metal-Enhanced Fluorescence (MEF) from large gold colloids is presented. Gold colloids, 40 and 200 nm diameter, were deposited onto glass substrates in a homogeneous fashion. The angular-dependent fluorescence emission of FITC-HSA, adsorbed onto gold colloids, was measured on a rotating stage which was used to evaluate MEF at all spatial angles. The emission intensity of FITC-HSA was found to be up to 2.5-fold brighter than the emission on bare glass substrates at an angle of 270 degrees. This is explained by the Radiating Plasmon Model, whereby the combined system, composed of the fluorophore and the metal colloids, emits with the photophysical characteristics of the fluorophore, after the excitation and the partial radiationless energy transfer between the excited states of the fluorophore and the surface plasmons of the gold colloids. The fluorescence enhancement was found to be higher with 200 nm gold colloids as compared to 40 nm colloids due to the increased contribution of the scattering portion of the 200 nm gold colloid extinction spectrum. These observations suggest that gold colloids could be used in MEF applications, offering more stable surfaces than the commonly used silvered surfaces, for applications requiring longer term storage and use.

**Keywords** Metal-enhanced fluorescence · Gold colloids · Fluorescein · Silver colloids · Light scattering · Angular-dependent emission · Radiating plasmon model · Surface-enhanced fluorescence · Radiative decay engineering · Plasmons · Plasmonics

## Abbreviation

HSA Human serum albumin  
MEF Metal-Enhanced Fluorescence  
RPM Radiating Plasmon Model

## Introduction

Metal-Enhanced Fluorescence (MEF), a phenomenon where the quantum yield and photostability of weakly fluorescing species are dramatically increased, due to proximity to free-electron rich metals, is becoming a powerful tool for the fluorescence-based applications of drug discovery [1, 2] high-throughput screening [3, 4] immunoassays [5] and protein-protein detection [1, 4, 6]. In this regard, many surfaces have been developed for metal-enhanced fluorescence [1–5], primarily based on silver nanoparticles, such as those comprised of silver islands [1, 5, 7], silver colloids [8], silver nano-triangles [9], silver nanorods [10] and even fractal-like silvered surfaces [11]. Several modes of silver deposition have also been developed, such as by wet chemistry [1, 9, 10], deposition by light [12] and electrochemically [13], on glass [5] and plastic substrates [14], HTS wells [3] and even electrodes [15].

Silvered surfaces perform very well in MEF applications when stored in water and used within a few days after preparation, and moreover, they can even be reused after heating at high temperatures such as 121°C [16] where most biological materials are no longer active. It was previously shown

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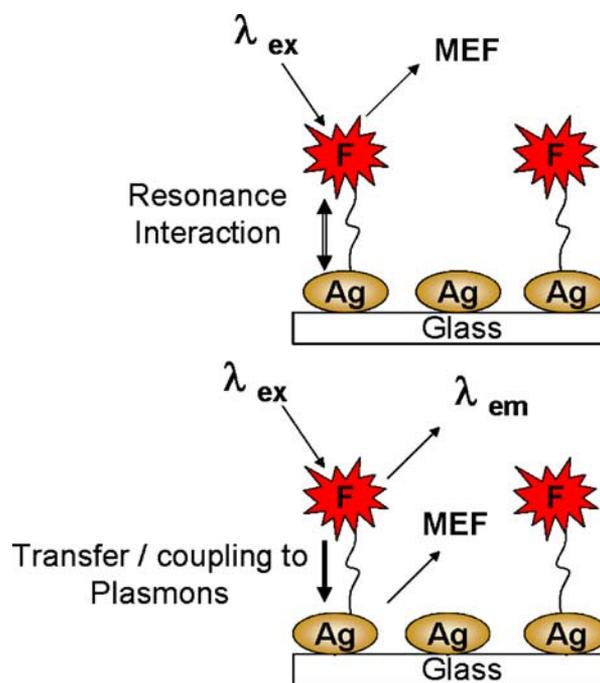
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that high temperature heating of silvered surfaces results in further increase of emission of fluorophores improving the sensitivity of bioassays [16]. However, the silvered surfaces do deteriorate after long storage times and are prone to oxidation at all temperatures, where the exposure to air at higher temperatures increases the oxidation process, which inherently limits their shelf-life for use in the longer term. In contrast, gold colloids are less prone to oxidation, and gold colloid-deposited surfaces are more stable, so that they can be stored (dry or in water) and used many months after their preparation.

Numerous reports [17–19] of the effects of gold colloids on fluorophores can be found in the literature, which show that the emission of fluorophores positioned in close proximity ( $< 10$  nm) to gold colloids up to 30 nm in diameter are “quenched,” due to the non-radiative energy transfer from the excited states of the fluorophores to the gold colloids. Until now, no observations have been made with regard to the enhancement of close proximity fluorophores fluorescence from gold colloids in solution or indeed on planar surfaces. This has been partly due to the use of relatively small gold colloids by many other workers [17–19], which have a dominant absorption component of its extinction spectrum, which is comprised of both absorption and scattering components [20, 21].

The extent of absorption and/or scattering of light by gold colloids also depend on their size. Typically, a 20 nm gold colloid will predominantly absorb light, where as larger sizes ( $> 100$  nm) predominantly scatter light at visible wavelengths [20, 21]. In a recent paper [22], it was shown that the light is scattered from large gold colloids (i.e., when the size of the nanoparticle is greater than 1/20th the wavelength of light) in an angular dependent fashion, where the scattered light intensity is highest at the observation angles of 90 and 270 degrees, and was zero at 0 and 180 degrees (angles are converted to match the angles used here), the intensity being proportional to  $\cos^2\theta$  as described by Rayleigh and Mie theory [22, 23]. It was also shown that the angular dependent light scattering at 270 degrees (forward scatter) increases with particle size: where 200 nm gold colloids had the highest degree of forward scatter [22].

So far, the observation of fluorescence enhancement has been limited to silver nanoparticles as reported in many papers and reviews of MEF [1–15]. In these reports, silver nanoparticles with different shapes [9–11] and sizes [8] were used, where typical enhancement values varied between 6–[14] to 3500-fold [13]. In all of these reports of MEF using silver nanoparticles, it was considered that the fluorophore is both excited and solely emits, the role of the surface plasmons being one of the near-field modification of the fluorophores far-field spectral characteristics, Fig. 1, i.e. a modification of the fluorophores intrinsic radiative decay rate [24]. Moreover, the description of the MEF phenomenon



**Fig. 1** Graphical representation of the original interpretation of Metal-enhanced Fluorescence (*Top*) and the current interpretation which is plasmon-assisted (*Bottom*). Both silver (Ag) and gold (Au) surfaces can be used. F, Fluorophore; MEF, Metal-enhanced Fluorescence

was later presented within a different framework, the Radiating Plasmon Model (RPM), whereby partial non-radiative energy transfer occurs from excited local fluorophores to the surface plasmon electrons in non-continuous films [16, 25], Fig. 1. The surface plasmons in turn, radiate (under certain conditions) the photophysical characteristics of the coupling fluorophores [25], in essence the system as a whole radiates. This interpretation of MEF has been facilitated by the previous findings of surface-plasmon field-enhanced fluorescence spectroscopy [26] and surface plasmon coupled emission [27], whereby fluorophores distal to a continuous metallic film can directionally radiate fluorophore emission at a unique angle from the back of the film. Remarkably, the plasmon coupled emission is nearly completely *p*-polarized, irrespective of the excitation polarization [27], strongly indicating that the emission is indeed coupled through the surface plasmons.

Given the well-known contribution of absorption to the extinction spectra for small colloids, and the fact that larger colloids are known to have substantial scattering components in their extinction, which is thought to facilitate MEF, then we hypothesized that larger gold colloids would also enhance close-proximity fluorescence signatures in an analogous manner to silver structures and not quench fluorescence, which is the more common observation [17–19]. Subsequently, in this paper, we report the first observation of MEF from gold colloids (40 and 200 nm), where the

emission of FITC-HSA on gold colloids is enhanced as compared to the emission on glass, a control sample. Subsequently, we believe that gold colloids will eventually find common place in MEF and other plasmonic applications.

## Materials and methods

### Materials

FITC-labeled human serum albumin (FITC-HSA) and premium quality APS-coated glass slides (75 mm × 25 mm) were obtained from Sigma-Aldrich. Gold colloids of 40 and 200 nm diameter were obtained from Ted Pella, Inc, CA. All chemicals were used as received.

### Methods

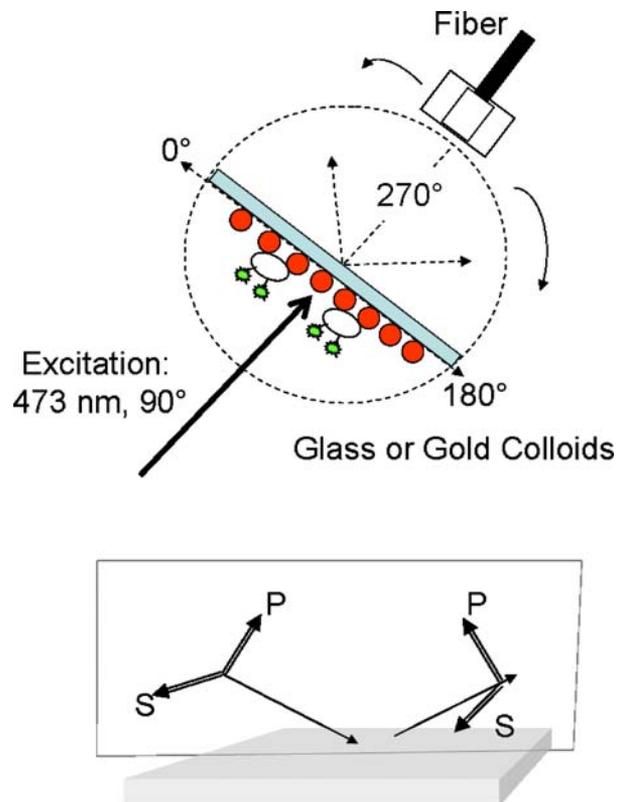
#### *Deposition of gold colloids onto APS-coated glass substrates*

The deposition of the gold colloids onto APS-coated glass slides was achieved by incubating the glass slides in a freshly prepared solution of gold colloids overnight. The APS-coated glass slides were coated with gold colloids due to the affinity between gold and amine groups, similar to the binding of silver to the amine groups of the surface polylysine [7], as demonstrated previously by our laboratories. The other half of the glass slides were left intentionally blank and served as the control experiments. The gold colloid deposited glass slides were rinsed with deionized water several times prior to the fluorescence experiments.

#### *Absorption, angular-dependent fluorescence measurements on gold colloids and on glass*

All absorption measurements were performed using a Varian Cary 50 UV-Vis spectrophotometer.

Binding the FITC-HSA to the gold colloids and to the glass surfaces was accomplished by incubating a 35  $\mu$ l solution of 10  $\mu$ M FITC-HSA on gold colloids and on glass for 30 min, followed by rinsing with PBS buffer to remove the unbound material. Both the gold colloids and glass surfaces were coated with the FITC-HSA, which is known to passively absorb to noble metal surfaces and form a  $\approx$  4 nm thick protein monolayer, allowing us to study the fluorescence spectral properties of non-covalent FITC-HSA complexes in the absence and presence of gold colloids. By equally coating the surfaces with FITC-HSA we were also able to determine the enhancement factor (benefit) obtained from using the gold, i.e. Intensity on gold/Intensity on glass, given

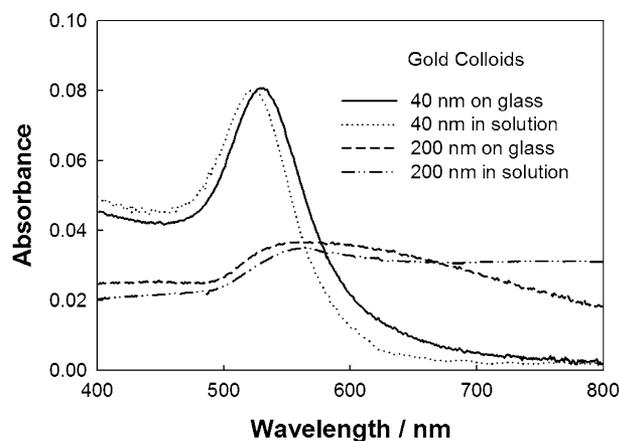


**Fig. 2** (Top)-Experimental setup. (Bottom)-Definition of “p” and “s” polarized light: p- and s-polarized light has the electric field polarized parallel and perpendicular to the plane of incidence, respectively

that both surfaces are known to have an  $\approx$  equal monolayer coverage.

Angular-dependent fluorescence spectra of FITC-HSA on glass and gold colloids were collected using an X-Y rotating stage (Edmund Optics), that was modified to hold a glass slide, with a fiber optic mount (Fig. 2-Top). The FITC-HSA on glass and gold colloids was excited with a polarized laser source, the second harmonic (473 nm) of the diode-pumped Nd:YVO<sub>4</sub> laser (compact laser pointer design, output power  $\approx$ 30 mW), at an angle of 90 degrees, and a neutral density filter was used to adjust the laser intensity. The excitation light was eliminated with an emission filter at 488 nm. The angular-dependent fluorescence emission peak of FITC-HSA and FITC-avidin through a polarizer were recorded at 517 nm using an Ocean Optics HD2000 spectrofluorometer. The emission intensity at the angles between 70 and 110° were not collected since the holder for the fiber optic assembly obstructed the view at those angles.

The real-color photographs of FITC-HSA on gold colloids and glass slides were taken with a Canon digital camera (S1-IS, 3.2 Mega Pixel, 10 × optical zoom) using the same long-pass filter that was used for the emission spectra.



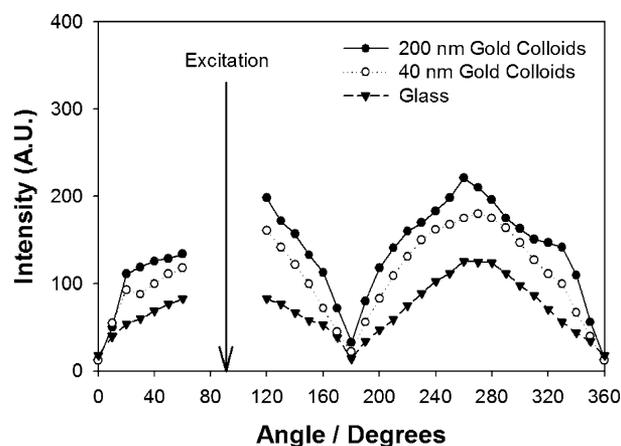
**Fig. 3** Absorption spectra of gold colloids in solution and gold colloids deposited onto glass slides

## Results and discussion

Figure 3 shows the absorption spectra of 40 and 200 nm gold colloids deposited on to glass substrates and in solution. The absorption spectrum for both 40 and 200 nm gold colloids deposited onto glass slides were similar to those in solution, indicative of a homogeneous deposition of gold colloids. The surface plasmon resonance (SPR) peak of 40 nm gold colloids showed a slight red shift from 525 nm in solution to 530 nm on the glass, where only minimal change in the SPR peak of 200 nm gold colloids was observed.

The homogeneous deposition of gold colloids, as achieved here, is important in the view of producing reproducible surfaces for the evaluation of the potential of gold colloids for MEF-based assays. It was previously shown that aggregated metal colloids deposited on planar surfaces provide better fluorescence enhancements [9–14], which is due to the increased electric field contribution from between the aggregated metal colloids. However, these surfaces lack the reproducibility necessary for the verification of MEF from gold colloids. Thus, in this paper it is those surfaces, where the gold colloids were deposited in a homogenous fashion that was considered.

Figure 4 shows the p-polarized emission intensity of FITC-HSA on glass substrates and on 40 and 200 nm gold colloids measured at 517 nm in an angular dependent fashion, i.e. over 360 degrees, where the excitation source was positioned perpendicular to the glass substrate, on the same side as the gold colloids and the fluorophores, c.f. Fig. 2. The emission intensities between the angles of 70 and 110° were not collected due to the obstruction by the fiber optic holder as explained in the Experimental section. The emission intensity of FITC-HSA on glass and gold colloids was the lowest at the angles of 0 degrees (equivalent to 360 degrees) and 180 degrees, and was the highest at 270 degrees (at the back of the glass substrates). The emission intensity



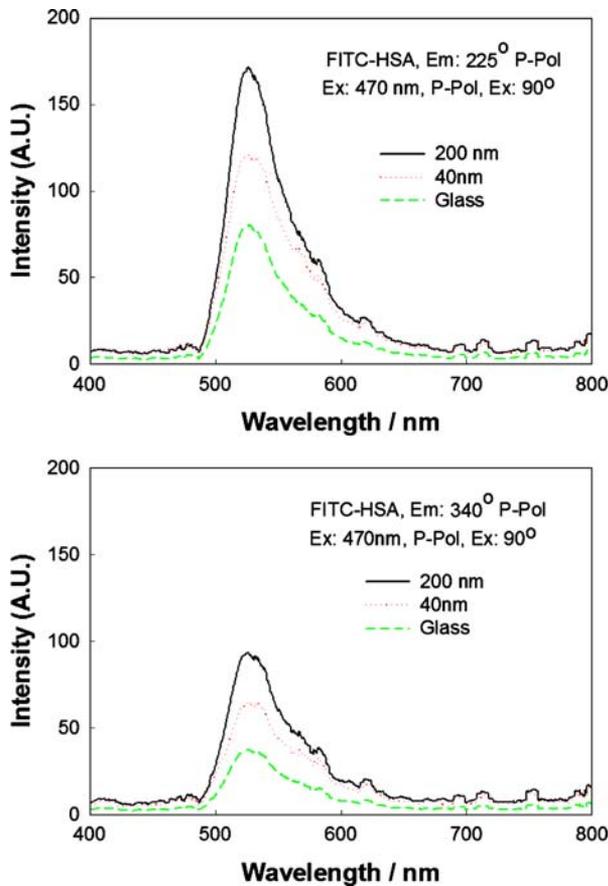
**Fig. 4** Angular dependent p-polarized fluorescence emission intensity (measured at 517 nm) for FITC-HSA on glass and gold colloids. Excitation was p-polarized and was at an angle of 90 degrees

at all angles was higher on 200 nm gold colloids than 40 nm gold colloids and glass, and the emission intensity at all angles was higher on 40 nm gold colloids than glass. That is, with respect to the emission intensity of FITC-HSA on glass, the emission intensity on 40 and 200 nm gold colloids were enhanced 1.75 and 2.5 times, respectively.

Figure 5 shows the p-polarized emission spectra of FITC-HSA on glass and gold colloids observed at 225 degrees and at 340 degrees. The emission spectra shown here are typical of those of FITC measured on glass substrates [3], and have a maximum emission peak of 517 nm. As also shown in Fig. 4, the emission intensity at 517 nm is the highest for 200 nm gold colloids as compared to 40 nm gold colloids and glass, and the emission intensity is indeed higher for 40 nm gold colloids than FITC-HSA coated glass.

A visual proof for the enhancement of fluorescence emission of FITC-HSA on gold colloids with respect to that of on glass is provided in Fig. 6, where the real-color photographs of fluorescence emission on glass and on 200 nm gold colloids taken at an angle of 225 and 340 degrees through an emission filter are shown. The fluorescence emissions on 200 nm gold colloids at both angles are much brighter than that of the glass substrate. Figure 6 also shows that the emission intensity of FITC-HSA on both glass and 200 nm gold colloids is higher when measured at 225 degrees than that of measured at 340 degrees.

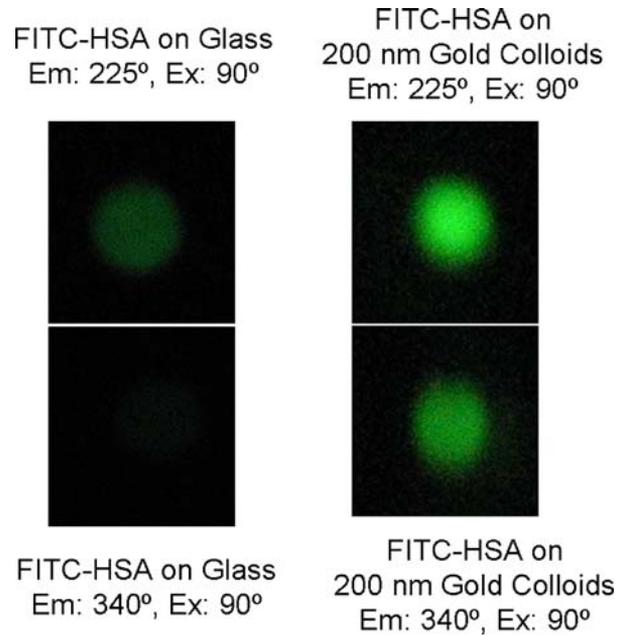
It is well established that the emission of fluorophores positioned in close proximity to gold colloids up to 30 nm in diameter [17–19] is “quenched,” due to the non-radiative energy transfer from the excited states of the fluorophores to the gold colloids. This energy is most probably then dissipated as heat. Since gold colloids less than 30 nm diameter result in quenching, and the larger gold colloids (larger than 40 nm) have increased forward scattering [22], and an increased scattering component in their extinction



**Fig. 5** P-polarized emission spectra of FITC-HSA on glass and gold colloids observed at 225 degrees (*Top*) and at 340 degrees (*Bottom*). Excitation source was p-polarized and was positioned at an angle of 90 degrees. A.U.-arbitrary units

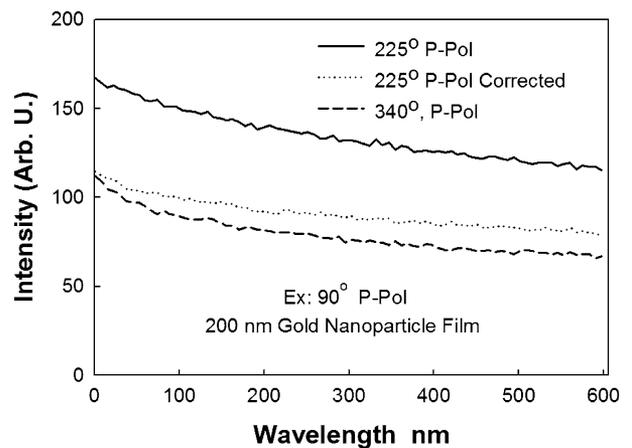
spectrum, then the gold colloids with a diameter of 40 nm and larger were predicted to enhance the fluorescence emission of fluorophores within close proximity. This hypothesis has been demonstrated with the data for both 40 and 200 nm gold colloids and FITC-HSA, c.f. Figs. 5 and 6. The use of fluorophores conjugated to a protein here, places the fluorophores within 4 nm of the gold colloid-coated surface. In this regard, gold colloid surface plasmons are excited in a non-radiative fashion [17, 18], which then efficiently scatter light, i.e., the photophysical properties of the fluorophore, c.f. Fig. 1-bottom. To the best of our knowledge, this is the first observation of an Plasmon-enhanced fluorescence from gold surfaces.

In many examples of fluorescence based sensing, it is fluorophore detectability that governs the utility and sensitivity of the sensing approach [28]. In general, the detectability of a fluorophore is determined by two factors: the extent of background emission from the sample and the photostability of the fluorophore. A highly photostable fluorophore, such as tetramethyl rhodamine [28] can undergo about  $10^6$



**Fig. 6** Real-color photographs of fluorescence emission of FITC-HSA on glass and 200 nm gold colloids taken through an emission filter (488 nm) at 225 and 340 degrees

excitation-relaxation cycles prior to photobleaching. While this can yield as many as  $10^4$  detectable photons per fluorophore it should be realized that the vast majority of fluorophores photodegrade after far fewer excitation-emission event cycles [28]. Subsequently, the photostability of FITC-HSA on the 200 nm gold colloids was tested at two different angles, 225 and 340 degrees. Figure 7 shows FITC—HSA emission as a function of time, excited at 473 nm and observed through a 488 nm long pass filter. The relative intensities of the plots reflect that more detectable photons can



**Fig. 7** Photostability of FITC-HSA from 200 nm gold colloids. Emission intensity (p-polarized) was measured at 517 nm and collected at 225 and 340 degrees

be observed per unit time from the 200 nm gold colloids at 225 degrees, as compared 340 degrees, where the integrated areas under the plots is proportional to the photon flux from the respective samples. By additionally adjusting the laser power to match the same initial steady-state intensities of the samples, Fig. 7, the FITC-HSA can be seen to be more photostable. This finding suggests that the lifetime of the FITC is shorter at 225 degrees, the FITC in essence spending less time on average in an excited state due to the fast non-radiative energy transfer to the plasmons, and therefore is less prone to photodestruction [1, 2], i.e. it is more photostable. Although, we were not able to measure the lifetime at 225 degrees, the evidence for a shortened lifetime of fluorophores in close proximity to metals, while the emission is still increased, has been shown many times before by our laboratories, using silver nanoparticles [1–15], but never in an angular-dependent fashion as shown here. The angular-dependent lifetime of FITC on gold colloids will be presented in a future paper.

## Conclusions

The first observation of MEF from gold colloids is reported. In this regard, 40 and 200 nm gold colloids were deposited, in a homogeneous fashion, on planar surfaces, which were used to validate MEF from gold colloids. The angular-dependent fluorescence emission of the FITC-HSA increased up to 2.5 times on gold colloids as compared to the emission from glass. The largest enhancement of fluorescence was obtained using 200 nm gold nanoparticles, observed at 270 degrees (or 180 degrees from the excitation source). These observations are explained by the Radiating Plasmon Model, whereby partial energy transfer from fluorophore to surface plasmons results in enhanced plasmon scatter, in addition to the free-space isotropic emission. The angular dependent nature of MEF, and its dependence on gold colloid size, is simply explained by the angular dependent nature of plasmon scatter [22], and the scattering vs. absorption contribution to the gold extinction spectrum.

These results indicate that gold colloid-coated surfaces can be used in fluorescence-based applications, such as in assays, where the fluorescence emission is enhanced and fluorophores became more photostable. Subsequently, we believe that gold colloids are likely to find common place in MEF as they are less prone to oxidation, as compared to silver colloids, and the fact that more surface chemistries have been developed for gold surfaces.

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