Maximizing Fluorescence Enhancement in Metal Nanoantenna Arrays for efficient bioanalytical devices

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Abstract. Our investigation focused on fluorescence enhancement mechanisms using metal nanoantennas with Alexa Fluor-647. By exploiting numerical modelling tools, we fabricated non-interacting Au nanodisc arrays on glass substrates, achieving a maximum fluorescence enhancement factor of 180 at an optimal spacer thickness of approximately 10 nm. Comparative analysis with bare glass substrates revealed significant improvements in excitation and emission dynamics, attributed to nanoscale field confinement and the Purcell effect. Time- and space-resolved photoluminescence measurements unveiled a distance-dependent interaction between the fluorophore and localized plasmons, modulated by thin polyelectrolyte monolayers, with prevalent radiative processes in samples exhibiting maximum signal.

1 Introduction

Fluorescence spectroscopy is a powerful analytical tool in biochemistry, medical diagnostics, and cellular imaging due to its sensitivity, specificity, simplicity, and cost-effectiveness compared to other techniques.[1] Particularly, it enables detection down to the single-molecule level, making it invaluable for various applications.[2] Fluorescent detection is favoured in clinical and environmental bioassays for its potential to achieve a low limit of detection and multiplexed detection using different fluorophore wavelengths. However, challenges like poor signal-to-noise ratios and photo-bleaching limit its sensitivity. To address this, Metal-Enhanced Fluorescence (MEF) techniques are pursued, leveraging near-field interactions between fluorophores and metallic nanoparticles to enhance emissions. Our study [3] explores the mechanism of fluorescence enhancement using metal nanostructures on planar substrates, revealing significant enhancements and potential applications in bio-sensing.

2 Method

Utilizing numerical simulations with COMSOL Multiphysics, we optimized geometric parameters of gold nanodisk arrays on glass substrates to enhance fluorescence across the visible and near-infrared spectrum. Our model accurately predicted Extinction Cross Section (ECS) and electric field distribution, validating experimental results. Through adjustments in metal thickness, disk diameter, and refractive index, we achieved resonance conditions for maximum plasmon-enhanced fluorescence (PEF). Fabrication involved hole-mask colloidal lithography on cleaned glass slides coated with PMMA, treated with oxygen plasma, and layered with positively charged PDDA polyelectrolyte and sulfate-stabilized polystyrene nanospheres. A 20 nm gold film was evaporated, and nanodisks were formed post-polystyrene removal. Spacer layers were deposited using polyelectrolyte multilayers with precise thickness control confirmed by AFM measurements.

3 Results and discussion

Finite-element numerical simulations were crucial in designing the plasmonic substrate, allowing systematic exploration of nano-antenna geometry for optimal performance. Variations in diameter and thickness were analysed to identify the most suitable combination for subsequent PEF experiments. The results highlighted an 80 nm diameter and 30 nm height as ideal, exhibiting plasmonic peaks at 610 nm in air and just under 650 nm in water, aligning well with fluorophore excitation and emission peaks. A low-cost technique involving polyelectrolyte multilayers was adopted to control the distance between the fluorophore and nanostructure, ensuring reproducibility and precise thickness control.

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Morphological characterization confirmed uniform nano-disk coverage with negligible agglomeration. Experimentally, plasmonic peak shifts were observed with increasing layer thickness, attributed to refractive index changes. PEF experiments demonstrated significant fluorescence enhancement, with maximum value (180) achieved with 11 PEM layers, confirming contributions from both excitation and emission enhancement mechanisms. The results were further supported by electric field distribution simulations, showing greater excitation enhancement contributions, particularly near the nanostructure edges. Fluorophore emission analysis and time-resolved photoluminescence measurements corroborated these findings, highlighting the importance of distance between the fluorophore and nanostructure.

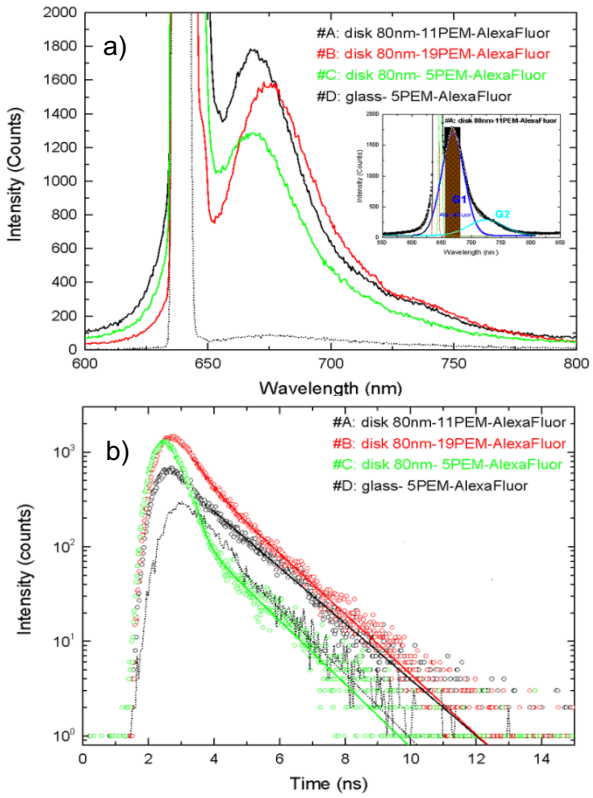

Fig. 1. a) PL spectra of Alexa fluor on different nanostructured metal substrates with different PEM layer numbers: #A (11 PEM), #B (19 PEM) and #C (5PEM). PL spectrum of 5 PEM reference sample is also shown (Alexa fluor on glass substrate with 5 PEM layers). Inset: Gaussian fit of PL signal of sample #A. b) PL decay signal registered at the maximum of PL emission of the Alexa fluor in sample #A, #B and #C.

Figure 1a presents the PL spectra of Alexa Fluor 647 deposited on Au nanostructured samples with varying numbers of PEM layers (5, 11, and 19 represented by green, red and black continuous lines) in a wet environment, along with the PL spectrum of the reference sample with 5 PEM layers (black dotted line). The influence of plasmon modes interaction is clearly observed, as the PL intensity of Alexa Fluor 647 is consistently higher in all metal samples compared to the corresponding reference sample with the same number of PEM layers. Our focus is on the G1 Gaussian emission band (inset fig. 1a), as its spectral position best aligns with the plasmon resonance, facilitating both excitation and fluorescence emission enhancement mechanisms. The TR-PL signal recorded in correspondence of the emission peak of G1 band, for each investigated sample (fluorophore on Au nanostructures) are reported in the figure 1b: the decay signals show bi-exponential behavior (continuous lines through the data). Table 1 presents the best fit decay time constants (τ) obtained from the analysis of PL decay dynamics, along with pre-exponential factors (A_{ref}) for both reference samples without nanodisks (exhibiting mono-exponential decay) and samples with nanostructured surfaces, demonstrating bi-exponential decay (Fig 1b), with A_f (fast) and A_s (slow) components. A_f and A_s represent the fraction of fluorophores recombining with fast and slow decay constants, respectively.

Table 1. Best fit pre-exponential factors and decay time constants (τ) resulting from analysis of the PL decay dynamics of #A, #B and #C samples of the related reference samples.

Sample	**#C 5PEM**	**#A 11PEM**	**#B 19PEM**
A_{ref}	250±2	220±2	194±2
t (ns) reference	1.36±0.01	1.34±0.01	1.53±0.01
A_f	550±10	800±200	360±20
t_f **(ns)**	0.31±0.01	0.11±0.01	0.44±0.03
A_S	130±6	464±6	600±20
t_S **(ns)**	1.41±0.03	1.46±0.01	1.37±0.01

The long decay time constants remain consistent across all samples, approximately 1.40 ns, closely resembling the values of the reference samples, indicating the PL signal from fluorophores not interacting with nanostructures. The short decay time constant, affected by the number of PEM layers, results from the shortened PL decay due to fluorophore-plasmon interaction on Au discs. The shortest lifetime, seen in the sample with the highest emission signal, suggests a strong association with the radiative process.

4 Conclusions

In this study, we explored metal nanodisc arrays as consistent platforms for plasmon-enhanced fluorescence, crucial for point-of-care testing devices. Using a common commercial dye, we deposited

monolayers at varying distances from Au nanostructures on planar substrates. Employing numerical modelling, we designed plasmonic substrates with non-interacting Au nano-disk arrays on glass using hole-mask lithography. Our findings demonstrated significant enhancement in excitation and emission dynamics due to nanoscale field confinement and the Purcell effect. Moreover, we controlled enhancing and quenching processes using thin polyelectrolyte monolayers. These results pave the way for improved sensitivity in fluorescence-based assays and efficient bio-photonic device development.

References

1. J. Ah Kim, D. J. Wales, G. Yang, *Prog. Biomed. Eng.* **2**, 042001, (2020)
2. J. Li, C. Li, R. F. Aroca *Chem. Soc. Rev*., **46**, 3962-3979, (2017)
3. D. Lospinoso, A. Colombelli, A. Cretì, M. Cesaria, M. Lomascolo, R. Rella, and M.G. Manera, *ACS Appl Opt Mater*, Article ASAP (2024)