Enhancement of the intrinsic fluorescence of adenine using aluminum nanoparticle arrays

Shankar K. Jha,1 Nassir Mojarad,2,3 Mario Agio,4,5 Jörg F. Löffler,1 and Yasin Ekinci1,2,*

1Laboratory of Metal Physics and Technology, Department of Materials, ETH Zurich, 8093 Zurich, Switzerland
2Laboratory of Micro- and Nanotechnology, Paul Scherrer Institute, 5232 Villigen-PSI, Switzerland
3Department of Mechanical and Process Engineering, ETH Zurich, 8803 Rüschlikon, Switzerland
4National Institute of Optics (CNR-INO) and European Laboratory for Nonlinear Spectroscopy (LENS), 50019 Sesto Fiorentino, Italy
5Present address: Laboratory of Nano-Optics, University of Siegen, 57072 Siegen Germany

*yasin.ekinci@psi.ch

Abstract: This study demonstrates the metal-enhanced fluorescence of adenine using aluminum nanoparticle arrays in the deep UV range. It achieves the reproducible intensity enhancement of intrinsic fluorescence up to 80 on well-defined aluminum nanoparticle arrays at 257 nm excitation. In addition to a high signal enhancement, a strong modification of the fluorescence emission spectrum of adenine is observed. This study illustrates that the label-free detection of DNA bases and proteins that have low intrinsic fluorescence and absorption bands in the deep UV range can be facilitated using aluminum nanostructures.

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References and links
1. B. Valeur, Molecular Fluorescence: Principles and Applications (Wiley-VCH, 2001).
2. J. R. Lakowicz, Principles of Fluorescence Spectroscopy (Springer, 2006).
3. J. W. Eastman, and E. J. Rosa, “The fluorescence of adenine: The effects of solvent and temperature on the quantum yield,” Photochem. Photobiol. 7, 189-201 (1968).
4. P. R. Callis, “Electronic states and luminescence of nucleic acid systems,” Ann. Rev. Phys. Chem. 34, 329-357 (1983).
5. D. Onidas, D. Markovitsi, S. Marguet, A. Sharonov, and T. Gustavsson, “Fluorescence properties of DNA nucleosides and nucleotides: A refined steady-state and femtosecond investigation,” J. Phys. Chem. B 106, 11367-11374 (2002).
6. C. F. Bohren and D. R. Huffman, Absorption and Scattering of Light by Small Particles (Wiley, 1983).
7. O. L. Muskens, V. Giannini, J. A. Sanchez-Gill, and J. Gomez Rivas, “Strong enhancement of the radiative decay rate of emitters by single plasmonic nanoantennas,” Nano Lett. 7, 2871 - 2875 (2007).
8. J. Munárriz, A. V. Malyshhev, V. A. Malyshhev, and J. Knoester, “Optical nanoantennas with tunable radiation patterns,” Nano Lett. 13, 444-450 (2013).
9. E. Fort, and S. Gresillon, "Surface enhanced fluorescence," J. Phys. D: Appl. Phys. 41, 013001 (2008).
10. C. D. Geddes, ed. Metal-Enhanced Fluorescence (John Wiley & Sons, 2010).
11. Y. Ekinci, H. H. Solak, and J. F. Löffler, “Plasmon resonances of aluminum nanoparticles and nanorods,” J. App. Phys. 104, 083107 (2008).
12. J. M. McMahon, G. C. Schatz, and S. K. Gray, “Plasmonics in the ultraviolet with the poor metals Al, Ga, In, Sn, Ti, Pb, and Bi,” Phys. Chem. Chem. Phys. 15, 5415-5423 (2013).
13. S. K. Jha, Z. Ahmed, M. Agio, Y. Ekinci, and J. F. Löffler, “Deep-UV surface-enhanced resonance Raman scattering of adenine on aluminum nanoparticle arrays,” J. Am. Chem. Soc. 134, 1966-1969 (2012).
14. D. O. Sigle, E. Perkins, J. J. Baumberg, and S. Mahajan, “Reproducible deep-UV SERRS on aluminum nanovoids,” J. Phys. Chem. Lett. 4, 1449-1452 (2013).
15. S. K. Jha, Y. Ekinci, M. Agio, and J. F. Löffler, “Towards deep-UV surface-enhanced resonance Raman spectroscopy of explosives: Ultrasensitive, real-time and reproducible detection of TNT,” Analyst 140, 5671-5677 (2015).
16. K. Ray, M. H. Chowdhury, and J. R. Lakowicz, “Aluminum nanostructured films as substrates for enhanced fluorescence in the ultraviolet-blue spectral region,” Anal. Chem. 79, 6480-6487 (2007).
has become a dominant technique in biochemistry and molecular genetics, where it plays a
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1. Introduction
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18. X. Jiao, and S. Blair, “Optical antenna design for fluorescence enhancement in the ultraviolet,” Opt.
17. F. Mahdavi, and S. Blair, “Nanoperture fluorescence enhancement in the ultraviolet,” Plasmonics 5, 169-
17. A. Maier, Plasmonics: Fundamentals and Applications (Springer, 2007).
24. E. C. Le Ru and P. G. Etchegoin, Principles of Surface-Enhanced Raman Spectroscopy (Elsevier, 2009).
25. W. A. Haynes and W. L. Barnes, “Plasmonic materials,” Adv. Mater. 19, 3771-3782 (2007).
26. C. L. Haynes, A. D. McFarland, L. Zhao, R. P. Van Duyne, and G. C. Schatz, “Nanoparticle Optics: The
importance of radiative dipole coupling in two-dimensional nanoparticle arrays,” J. Phys. Chem. B 107, 7337 – 7342 (2003).
27. J. Sung, E. M. Hicks, R. P. Van Duyne and K. G. Spears, “Nanoparticle spectroscopy: Plasmon coupling in
finite sized two-dimensional arrays of cylindrical silver nanoparticles,” J. Phys. Chem. C 112: 4091 – 4096 (2008).
28. J. Parsons, E. Hendry, C. P. Burrows, B. Auguié, J. R. Sambles and W. L. Barnes, “Localized surface-
plasmon resonances in periodic nondiffracting metallic nanoparticle and nanohole arrays,” Phy. Rev. B 79, 073412 (2009).
29. J. Olson, A. Manjavacas, L. Liu, W.-S. Chang, B. Foerster, N. S. King, M. W. Knight, P. Nordlander, N. J.
Halas, and S. Linka, “Vivid, full-color aluminum plasmonic pixels, PNAS 111, 14348–14353 (2014).
30. N. Akbay, J. R. Lakowicz, and K. Ray, “Distance-dependent metal-enhanced intrinsic fluorescence of
proteins using polyelectrolyte layer-by-layer assembly and aluminum nanoparticles,” J. Phys. Chem. C 116, 10766-10773 (2012).
31. H. Mishra, B. L. Mali, J. O. Karolin, A. I. Dragan, and C. Geddes, “Experimental and theoretical study of
the distance dependence of metal-enhanced fluorescence, phosphorescence and delayed fluorescence in a
single system,” Phys. Chem. Chem. Phys. 15, 19538-19544 (2013).
32. P. Anger, P. Bharadwaj, and L. Novotny, “Enhancement and Quenching of Single-Molecule Fluorescence,”
Phys. Rev. Lett. 96, 113002 (2006).
33. L. Zhao, T. Ming, H. Chen, Y. Liang and J Wang, “Plasmon-induced modulation of the emission spectra
of the fluorescent molecules near gold nanorods,” Nanoscale 3, 3849-3859 (2011).
34. C.-Y. Wu, C.-L. He, H.-M. Lee, H.-Y. Chen, and S. Gwo, “Surface-plasmon-mediated photoluminescence
enhancement from red-emitting InGaN coupled with colloidal gold nanocrystals,” J. Phys. Chem. C 114, 12987-12993 (2010).
35. E. C. Le Ru, P. G. Etchegoin, J. Grand, N. Félidj, J. Aubard, and G. Lévi, “Mechanisms of spectral profile
modification in surface-enhanced fluorescence,” J. Phys. Chem. C 111, 16076–16079 (2007).
36. M. Ringler, A. Schwemer, M. Wunderlich, A. Niehl, K. Kürzinger, T. A. Klar, and J. Feldmann, “Shaping emission spectra of fluorescent molecules with single plasmonic nanoresonators,” Phys. Rev. Lett. 100, 203902 (2008).
37. R. M. Bakker, H. –K. Yuan, Z. Liu, V. P. Drachev, A. V. Kildishev, V. M. Shalaev, R. H. Pedersen, S.
Gresillon, and A. Boltasseva, “Enhanced localized fluorescence in plasmonic nanoantennas,” Appl. Phys.
Lett. 92, 043101 (2008).
38. Qiang Li, Stefan Seeger, “Label-free detection of protein interactions using deep UV fluorescence lifetime
microscopy,” Anal. Biochem. 367, 104–110 (2007).
39. P. Schulze, M. Ludwig, F. Kohler, and D. Belder, “Deep UV laser-Induced fluorescence detection of
unlabeled drugs and proteins in microchip electrophoresis,” Anal. Chem. 77, 1325-1329 (2005).

1. Introduction
Fluorescence-based techniques are essential methods for detecting, monitoring, and studying
molecules, with widespread applications in various fields including biosciences, chemistry,
medicine, and materials science [1,2]. Given its high sensitivity, fluorescence spectroscopy
has become a dominant technique in biochemistry and molecular genetics, where it plays a
pivotal role in DNA sequencing [1,2]. However, most nucleic acids and nucleotides are
considered virtually non-fluorescing because their intrinsic fluorescence quantum yield (Q0) is
very low. The quantum yield of DNA bases, for example, is often reported to be on the order

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of $10^4$ [3-5]. Therefore, specific labeling of these molecules by using fluorescent dyes becomes necessary for DNA sequencing and genetic analysis. Using such specific labels, however, is labor-intensive, time consuming and hence expensive. Similarly, label-free detection of proteins and peptides using fluorescence spectroscopy can be challenging. Only a few amino acids, such as tryptophan and tyrosine, have reasonable fluorescence quantum yields [2], and therefore proteins and peptides lacking these have very low intrinsic fluorescence.

The total quantum yield of fluorophores can be increased by bringing them into close proximity with metallic nanostructures. In the presence of a metal nanostructure, the excitation intensity can be increased by enhancing the electromagnetic (EM) field near the metal surface, which occurs due to the excitation of localized surface plasmon resonances (LSPRs) [6]. The metal can also increase the radiative emission rate and therefore the quantum yield of the fluorophore [7]. Moreover, the metal nanostructure can modify the radiation pattern, which can result in increased collection efficiency [8]. Together, these three effects may dramatically increase the fluorescence signal and enable detection of low-yield fluorophores. Collectively, these phenomena are referred to as surface-enhanced fluorescence (SEF) [9] or metal-enhanced fluorescence (MEF) [10].

In this study we report on the enhancement of intrinsic fluorescence of the DNA base adenine using Al nanoparticles. Our intention is to realize highly sensitive and reproducible detection of the biomolecule without using any dye labels. Adenine, like other DNA bases and many proteins, exhibits electronic absorption in the deep-ultraviolet (DUV) wavelength region and has a broad peak at around 260 nm [2]. To enable excitation enhancement, the metal substrate should support LSPRs in this wavelength region. It is well known that Al is the best choice for plasmonic applications in the ultraviolet (UV) and DUV regions [11-15]. Indeed, several reports have emerged on the use of Al substrates to enhance the fluorescence of various fluorophores. Ray and co-workers [16] demonstrated an enhanced fluorescence from the DNA base analogue 2-aminopurine deposited on Al nanostructured films. Corrugated Al nanostructures were employed to suitably modify fluorescence emission direction and increase collection efficiency, and hence detection sensitivity [17,18]. It has been predicted that up to 100-fold enhancement in the quantum yield can be achieved by using Al plasmonic antennas that suitably modify the local field and the emission pattern from the fluorophore. Theoretical calculations by Chowdhury and co-workers [19-21] indicate that using appropriately designed nanoparticles can generate highly amplified fluorescence emission from molecules and allow label-free detection of biomolecules. The deployment of surface plasmon polariton modes on Al thin films for MEF has also been demonstrated [22]. Hence, it is clear that Al nanostructures are suitable substrates for MEF applications in the DUV. In most experimental reports, however, rough thin films (i.e. island-films) obtained by controlled evaporation of Al have been used as substrates [16, 19-21]. Such nanostructures are inhomogeneous and have very broad and weak LSPRs. Therefore, they are hardly reproducible and generate only a modest signal enhancement. In the following we report on a systematic investigation of MEF in the DUV range in the context of well-defined plasmonic nanostructures, and show the potential of DUV-MEF for detecting a biologically important molecule with low intrinsic fluorescence.

2. Experimental section

2.1 Nanostructure fabrication

In this study Al nanoparticle (NP) arrays were fabricated by electron-beam lithography (EBL) on fused silica (FS) substrates (Suprasil, Heraeus). This method offers great control over the size, shape and periodicity of the fabricated nanostructures. The FS substrates were spin-coated with 100-nm-thick poly(methyl methacrylate) (PMMA 950k, diluted to 2% in ethyl lactate), and then baked at 170 °C for 3 minutes. This was followed by thermal
deposition (Balzers) of a 5-nm-thick Cr layer over the resist layer to avoid sample charging during the EBL process. The samples were then exposed with an EBL tool of 100 keV (Vistec EBPG5000Plus). After exposure, the chips were dipped in Cr-etchant solution for 30 s to remove the top Cr layer and subsequently rinsed with deionized water. Resist development was carried out using a 1:3 solution of methyl isobutyl ketone (MIBK) and isopropyl alcohol (IPA), resulting in holes in the PMMA film. E-beam assisted thermal evaporation (Univex 500, Oerlikon Leybold Vacuum) of Al through the hole-arrays and resist lift-off in acetone produced Al NP arrays on FS substrate.

Fig. 1. SEM images of the NP arrays fabricated using EBL process. Low-magnification (a) and high-magnification (b, c) SEM images of Al NP arrays. The block-wise arrangement of the NP arrays with 5 × 5 matrix of such arrays (partly shown in (a)) are designed on the substrate. In a row, i.e. from left to right, the NP diameters increase in steps of 25 nm. Along the column the period increases in steps of 25 nm. We note that the field in the first column of the lower row did not yield a uniform array of NPs due to the fabrication process, and such fields are omitted in further investigations.

Al NP arrays of various periods and diameters were fabricated. A single field of identical NPs had dimensions of 100 × 100 µm². NPs were arranged in a square lattice. A matrix of 5×5 fields were fabricated with different diameters (d) and periods (P). In a row, in the fields of NP arrays the particle diameter varied from 50 nm to 150 nm with incremental steps of 25 nm. Along the column, the period of the array increased from 150 nm to 250 nm in steps of 25 nm. The particle height was 70 nm. Figures 1(a)–1(c) show SEM images of NP arrays obtained with various SEM magnifications. As shown in Fig. 1(a), the fields are arranged in blocks with a 10-µm separation between the fields. The resulting Al NPs have slightly different diameters than the designed diameter in EBL due to proximity effects. The actual diameters of NPs of different fields were obtained by SEM. Some of the designed fields, such as those with small periods and large diameters, could not be achieved via the nanofabrication steps outlined above.

2.2 Sample preparation

For fluorescence measurements a stock solution of 1 mM adenine was prepared by dissolving 135.13 mg of adenine (99.5% purity, Sigma-Aldrich) in a liter of ultrapure deionized water
(18.2 MΩ cm resistivity). Because the solubility of adenine in water is very low, the mixture was stirred overnight with a magnetic stirrer to achieve a uniform solution. For MEF applications, the 1 mM solution was diluted further to 0.05 mM concentration. The samples were prepared by drop-coating 2 µl of 0.05 mM adenine solution on the substrates. The diameter of the resulting circular area to which the solution spread was ~3 mm in diameter. Thus an area much larger than the active measurement region (< 1 mm²) was covered by the adenine solution. As discussed below, the drop-coating method provides a uniform spread of the molecules on the substrate except for the so-called coffee-ring pattern, which is caused by accumulation of molecules at the drying boundaries.

2.3 Measurement setup

The measurements were performed using a home-built optical setup in back-scattering geometry with a continuous wave 257 nm laser excitation (Innova 90 C FreD, Coherent Inc.). The simple combination of an UV focusing objective (NA = 0.4, f = 10 mm; Microspot, Thorlabs) and tube lens (f = 200 mm; Mitutoyo) served as a functional UV-microscope with an effective magnification of 20×. A grating monochromator (SpectraPro2500i, Princeton Instruments) coupled with a UV optimized, back-illuminated, liquid-nitrogen-cooled CCD detector (PIXIS: 2K/BUV, Princeton Instruments) served as an imaging spectrometer. A low dispersion grating (150 lines/mm) was used to enable detection over a broad spectral range. A Rayleigh edge filter (Semrock Inc.) – a long-pass optical filter – with an optical density > 6 at 257 nm was used to suppress the excitation wavelength. The laser spot size at the sample was measured as ~1 µm. The incident optical power was ~1 mW at the sample. This setup made possible the spectral measurements of plasmon absorption and fluorescence as well as the imaging needed to navigate to different fields on the sample.

3. Results and discussion

3.1 Plasmon resonance of Al NP arrays

The fabricated Al NP arrays exhibit strong LSPR modes over a broad wavelength range including UV and DUV spectral regions. The tunability of the LSPRs by varying the two NP parameters, i.e. diameter (d) and periodicity (P), is shown in Figs. 2 and 3, respectively. It is observed that the arrays exhibit two prominent LSPR modes. The one at shorter wavelengths correspond to the high-energy quadrupolar LSPR mode and the one at longer wavelengths to the lower energy dipolar mode.

Figures 2(a) and 2(b) show the dependence of the LSPRs on particle diameter for fixed periodicities. While the value of d varies from 50 to 150 nm, the value of P is 200 nm and 250 nm in Figs. 2(a) and 2(b), respectively. It is seen clearly that as the value of d increases there is a red-shift of the resonance positions and a broadening of the resonance bands. The red-shift is attributed to the retardation effects that correspond to the change in amplitude and phase of the incident EM field across the nanoparticle [23-25]. The shift is more pronounced for the dipolar mode. In addition, the width of the dipolar mode increases with increasing NP diameter due to increased radiative damping. For a given value of P as the value of d increases, the extinction spectrum becomes more complex with excitation of multiple modes. In Fig. 2(b), for example, NP arrays with diameters of 125 nm and 150 nm exhibit weak resonances of between 325 nm and 400 nm. The extinction efficiency of the quadrupolar mode is highest for the largest particle diameter and vanishes with decreasing particle size because the higher-order modes are not sustained or become weaker with decreasing particle size.

Figures 3(a) and 3(b) show the dependence of the extinction spectra on the NP periodicity. While keeping the particle diameter fixed at 75 nm and 100 nm in Figs. 3(a) and 3(b), respectively, the value of P is increased from 150 nm to 250 nm in steps of 25 nm. It is observed that for the same value of d there is a red-shift and narrowing of the resonance bands
with increasing $P$. The effect is more prominent for the dipolar LSPR mode. As reported by Haynes [26] and others [27, 28], the red-shift of resonance with increased periodicity is due to the long-range radiative dipole coupling between the nanoparticles in a periodic array. With decreasing periodicity, the distance between the individual nanoparticles decreases and thereby the radiative dipole coupling, which leads to shift of the modes to higher energies, increases. To this end, we show that the LSPRs modes in Al NP arrays can be tuned across a broad range.

Fig. 2. Effect of NP diameter ($d$) on the LSPR modes. Extinction spectra of Al NP arrays with the periods (a) 200 nm and (b) 250 nm, and various particle diameters.

Fig. 3. Effect of NP period ($P$) on the LSPR modes. Extinction spectra of Al NP arrays with the diameters (a) 75 nm and (b) 100 nm, and various particle periodicities.

As seen in Figs. 2 and 3, Al nanoparticles are rich in plasmon resonances and show a great tunability of the LSPR modes. Similar to the strong, tunable, and multiple resonances of Al reported in the visible range [29], we show here that Al can also be tuned in the DUV range and it is relatively simple to match the laser excitation wavelength of 257 nm. The EM near-field enhancement at this wavelength can lead to increased excitation intensity and thus may give rise to a significant increase in fluorescence quantum yield.

3.2 MEF of adenine

Figure 4(a) shows an optical image of a NP array and its adjoining area, where adenine was drop-coated. It is seen that after the evaporation of solvent a coffee-ring type pattern remained on the substrate, whereas the rest of the surface is covered with a uniform layer of adenine. Reference fluorescence measurements, i.e. fluorescence spectra of adenine thin film without enhancement, were made on bare areas avoiding the inhomogeneous coffee-ring pattern.

Figure 4(b) shows the fluorescence spectra of adenine acquired from such a bare region on the substrate. Each signal was acquired for 10 ms, because short data acquisition times are advantageous due to the strong radiation damage in the DUV range. 25 spectra were obtained by moving the sample stage in steps of 20 µm using a piezo-stage. All the acquired spectra are plotted in gray. As seen, it is impossible to detect the fluorescence signal in the spectra.
dominated by the noise. The highlighted spectrum in black represents the mean of the 10-point adjacent average of 25 individual spectra. It can be seen that a faint signal emerges from the noise background upon taking a mean of all the spectra. Two broad peaks at about 320 nm and 450 nm can barely be identified, which are the emissions band of adenine. The sharp peak at about 260 nm is the Raman peak of the FS substrate [13]. Two horizontal lines in the center are marked for better visualization of the data.

In Fig. 4(c) the MEF spectra acquired on an Al NP array are plotted. 25 spectra were acquired in a manner similar to the reference measurements made on the bare substrate. Once again, all the spectra are plotted in gray and their mean is highlighted in red. It is seen that despite a relatively small fluctuation in the measured signal, overall the MEF intensity is well reproduced for individual acquisitions, indicating the good uniformity of both adenine concentration and the enhancement factor for Al NPs. The prominent emission band lies in the region between 310 nm and 350 nm, with a maximum close to 325 nm. A second emission band of adenine can also be seen in the region between 400 nm and 500 nm. A small and sharp peak at about 275 nm in this case results from the Raman signal of adenine [13].

Comparing Figs. 4(b) and 4(c), it can be seen that while the height of the fluorescence peak is barely 2 counts, the MEF intensity is clearly much higher. To highlight the effect further, the mean values of the measurements are plotted together in Fig. 4(d) for comparison. A clear enhancement of the fluorescence signal is observed for the molecules deposited on Al NPs with periodicity of 225 nm, particle diameter of 142 nm and the particle height of 70 nm. The effective height of the MEF peak at around 325 nm is ~160 counts after subtracting the background. Thus a relative fluorescence intensity enhancement of 80 is achieved by using Al NPs. The increase in intensity is roughly one order of magnitude higher in comparison to the reported experimental fluorescence enhancement factors using Al island-films [16,19].

It is known that the metal-fluorophore separation plays an important role in the observed enhancement factor. Inactive, i.e. dielectric, spacer layers are often employed to control the distance between the metal and fluorescent molecules [16, 30-32]. In our studies, the adenine layer is directly coated on the NPs without an additional spacer layer. The native oxide layer on the Al NPs acts, however, as a natural spacer layer and the thickness of the oxide is about 3-5 nm [11].
Fig. 4. (a) Coffee-ring pattern formed after drop-coating of adenine solution on the substrate. (b) Fluorescence spectra of adenine drop-coated on a bare area on the quartz slide. (c) MEF spectra of adenine deposited on an Al NP array. (d) Comparison of the mean intensities of the fluorescence and MEF spectra. The spectra were acquired after drop-coating the sample with 0.05 mM adenine. The NP array has a periodicity of $P = 225$ nm, particle diameter of $d = 142$ nm and the particle height of $h = 70$ nm. A relative intensity enhancement of ~80 is observed.

3.3 Spectral modification

Apart from the fluorescence signal enhancement, we observed significant variation in the MEF spectra of adenine from one NP array to the other. Figure 5 shows the effect of particle diameter on the MEF intensity and spectrum while the period is kept constant. Measurements on NP arrays with two different periodicities are presented. In Fig. 5(a) the periodicity is 200 nm and in Fig. 5(b) it is 225 nm. The experimental conditions are the same as in previous cases. The means of 25 spectra acquired on each array are plotted. From these figures it can be observed that not only do the fluorescence intensities vary, but the emission spectra also exhibit strong modifications. A similar effect is observed when the periods are varied while keeping the particle diameter roughly the same. The results are shown in Figs. 6(a) and 6(b), where in each panel the periods increase in steps of 25 nm while there is no significant change in the diameter values. Spectral modifications in terms of shift in the peak position, spectral broadening, and appearance of new peaks are clearly observed.

To understand the spectral modification in MEF further, extinction spectra of the NP arrays were compared with the fluorescence signals acquired from them. Figure 7 presents the SEM images, extinction spectra and MEF spectra of 100 nm diameter Al NPs of four different periodicities (indicated above each column). The corresponding extinction and MEF spectra are plotted under each panel. The wavelength axes are the same in all extinction and MEF panels. From left to right a significant variation in the fluorescence intensity and spectral shape can be seen. While the fluorescence intensity is enhanced by each NP array, the spectral...
changes introduced by the arrays are unique. This indicates a strong interplay between the fluorophore and the optical properties of the metal nanostructures and thus a substantial and complex influence on the resulting emission spectrum. Unlike the Raman signal enhancement by Al NP arrays, which depends predominantly on the EM near fields [13, 14], the phenomenon of MEF is caused by multiple mechanisms of interaction of the fluorophore with the NP. Near-field enhancement increases the excitation of molecules, whereas the emission is enhanced or quenched due to the increase in the quantum yield of radiative and non-radiative transitions. Therefore the plasmon resonance of the NP at both excitation and emission wavelengths plays a significant role in MEF. The modification of the fluorescence spectrum of various fluorophores due to their plasmonic coupling with metal nanostructures has been documented previously [33-37]. It has also been reported that the spectral variation is generated by modification of the energy levels of the emitter in the presence of the metal nanostructure [35, 36]. All these factors generate a strong modification of the fluorescence spectrum, along with its enhancement. Further theoretical and experimental investigations should thus be undertaken to understand the mechanisms of spectral modification induced by the plasmonic nanostructures, including variations in the collection efficiency for different LSPR modes.

Fig. 5. MEF spectral dependence on particle parameters. The values of period ($P$) and diameter ($d$) are indicated in each panel. In (a) and (b) the period remains constant while the particle diameter is varied.

Fig. 6. MEF spectral dependence on particle parameters. In both (a) and (b) the period increases in steps of 25 nm, while the diameter does not change significantly.
Fig. 7. Comparison of LSPR and fluorescence spectra of adenine deposited on NP arrays of various periods. In the top row the representative SEM images of the arrays are presented. A gradual increase in interparticle separation is clearly visible from these images. The scale bar in each panel is 500 nm. The middle and bottom rows show, respectively, the extinction spectra of the arrays and MEF spectra of adenine acquired from them. A gradual red-shift of the plasmon resonance peaks in the extinction spectra with increasing particle periodicity is seen. Significant changes in the MEF spectra result from the plasmonic coupling of the fluorophore with the metal.

7. Conclusions

In summary, designed Al plasmonic nanostructures show strong MEF in the DUV range. We observed up to 80-fold enhancement of intrinsic fluorescence of adenine with Al NP arrays at 257 nm excitation. The observed enhancement is about one order of magnitude higher than the values found in most experimental reports, in particular those on MEF in the DUV range [16, 19]. Employing well-defined Al NP arrays facilitates reproducible and high enhancement factors. The great tunability of Al NP arrays means that the plasmon resonances are highly sensitive to geometry and size, and therefore ensembles of Al NPs of small size and geometry dispersion are crucial to obtain sharp and pronounced plasmon resonances. A significant advantage of Al NPs for MEF is the fact that multiple resonances of Al NPs generate high enhancement factors by increasing both excitation and emission efficiency. In this study we systematically changed the geometrical parameters of periodicity and diameter of NP arrays. The thickness of the NPs and NP-to-fluorophore distance (defined by the natural oxide layer) were kept constant and further enhancement may be achieved by rigorous optimization of these parameters. In particular, optimization of the NP-to-fluorophore distance can produce greater enhancement factors, and it is well known that fluorescence enhancement is very sensitive to this parameter. This distance can be increased by depositing additional oxide layers using atomic layer deposition or by annealing the substrates, resulting in an increase in the thickness of the oxide layer.

We have also shown that plasmonic coupling between the fluorophore and the Al NP arrays generates spectral modification of the fluorescence signal. Al NPs provide strong and highly tunable plasmon resonances and can therefore be used in further analysis to understand the phenomenon of MEF. Detailed studies on the dependence of fluorescence lifetimes and their wavelength dependence with tunable plasmonic resonances will provide a better insight into the underlying mechanisms of MEF.
In general, we have demonstrated that MEF in the DUV range with Al NP arrays is a powerful method for increasing the detectability of molecules with low intrinsic fluorescence. Although this study was limited to adenine, many biomolecules share similar optical properties, i.e. low fluorescence quantum efficiency and absorption band in the DUV range. Fluorescence microscopy in the DUV range has already been demonstrated as an effective method for label-free detection of drugs and proteins as well as of protein interactions [38, 39]. Al NPs can be easily integrated into chip-based analytical methods [38], and can thereby combine the simplicity and throughput of these methods with great sensitivity. It should be also noted that Al, unlike commonly used plasmonic materials, is a low-cost material and compatible with mainstream nanofabrication processes, making it more feasible for real applications. For this reason MEF in the DUV range with Al NP arrays has great potential for detecting chemicals and biomolecules, such as small aromatic compounds, DNA bases, amino acids, proteins, and peptides.

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