Plasmonic Antennas for Directional Sorting of Fluorescence Emission

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ABSTRACT: Spontaneous emission of fluorescent molecules or quantum dots is radiated along all directions when emitters are diluted in a liquid solution, which severely limits the amount of collected light. Besides, the emission direction does not carry any useful information and cannot be used to sort different molecules. To go beyond these limits, optical antennas have been recently introduced as conceptual tools to control the radiation properties for nanoemitters fixed on a substrate. Despite intense recent research, controlling the luminescence directivity remains a challenge for emitters with random positions and orientations, which is a key for several biomolecular screening applications. Here, we present full directional control of the fluorescence emission from molecules in water solution by an optical antenna made of a nanoaperture surrounded by a periodic set of shallow grooves in a gold film. For each emission wavelength, the fluorescence beam can be directed along a specific direction with a given angular width, hereby realizing a micrometer-size dispersive antenna. We demonstrate the fluorescence beaming results from an interference phenomenon and provide physical optics guidelines to control the fluorescence directivity by tuning the groove—nanoaperture distance. This photon-sorting capability provides a new approach for high-sensitivity screening of molecular species in solution.

KEYWORDS: Nanoantenna, plasmonics, metal nanoaperture, fluorescence enhancement, grating

Detecting the fluorescence emission of single molecules or quantum dots in solution is widely exploited in several fields of analytical chemistry, genomics, molecular biology, or medicine. Optimizing the overall photon detection efficiency and using a small sensing volume are both crucial points to achieve single-emitter detection with proper signal-to-noise ratio. To improve the interaction between a single quantum emitter and the far-field radiation, photonic structures known as optical antennas are the subject of intense research. Optical antennas have been demonstrated to squeeze light into nanoscale volumes, enhance the excitation and emission rate of individual emitters, tune the luminescence spectrum, and control the directivity properties of emitted light. However, these antennas work for individual emitters fixed on a substrate with a specific dipole orientation. Developing an optical antenna to control the fluorescence directivity in the case of an ensemble of molecules with random dipolar orientation remains an open challenge. An elegant way to concentrate the fluorescence emission into a narrow angular cone takes advantage of the surface plasmon coupled emission on a thin metal film in Kretschmann configuration. While providing angular divergences below 10°, this method is however limited by moderate signal enhancement per molecule and large sampling volumes. Hence, an optical nanoantenna approach appears necessary to further improve the practical range of biophotonic applications.

Single nanoapertures surrounded by periodic grooves are of great interest to improve the detection of single quantum emitters in solution. This antenna design merge the light localization from the nanoaperture with the extended near to far-field conversion capabilities from the concentric grooves. The central nanoaperture reduces the sampling volume and enables single molecule analysis at high concentrations with enhanced excitation and emission rates. The periodic grooves act like an antenna to further concentrate the electromagnetic energy at the central aperture and to control the directivity of the radiated light. The combination of these two properties has lead recently to the demonstration of fluorescence enhancement factors up to 120 fold while using a nanoaperture surrounded by 5 circular grooves. The same nanoantenna demonstrated simultaneously narrow emission radiation pattern in a cone of ±15° in the direction normal to the sample. However, a complete physical description of the phenomenon leading to fluorescence beaming is still lacking, as well as a thorough exploration of the design parameter space to control the fluorescence directanly.

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Here, we propose a class of optical antennas to control the fluorescence emission directivity for molecules in solution. The key result is that for each emission wavelength the fluorescence beam can be directed along a specific direction with a given angular width, hereby realizing a micrometer-size dispersive element. With photodetectors placed underneath the antenna, this device would realize a miniature spectrometer. Remarkable tunability over the fluorescence directivity is achieved by varying a single design parameter, which is the groove–nanoaperture distance \( a \) (Figure 1). We provide physical optics guidelines to adequately select this parameter, the key concept being to set adequately the phase relationship between the emission from the nanoaperture and the surface radiation scattered by the grooves. This manuscript provides a complete characterization of the interference phenomenon leading to fluorescence beaming, both experimentally and theoretically. Very importantly for biophotonic applications, we demonstrate fluorescence directivity control for an ensemble of molecules with random orientation and position inside the central aperture. The fluorescence radiation patterns are thoroughly analyzed and calculated back to average signal per molecule, quantifying the fluorescence count rate enhancement found for each emission angle. The photon-sorting capability combined to fluorescence enhancement factors of several fold provide a new approach for efficient spectral detection of molecular species in solution.

Nanoapertures with concentric grooves are realized by focused ion beam (FIB) milling. Figure 1 presents our experimental configuration together with scanning electron microscope views of our samples. The main parameter of interest in this study is the distance \( a \) from the center of the aperture to the first groove, as shown in Figure 1a. Throughout this study, all the other design parameters are kept constant and have been optimized according to the characterization for the transmission process: \(^{39,41}\) the aperture central diameter is 140 nm, the groove period is 440 nm, the groove width is 200 nm, and groove depth 65 nm. For the experimental data reported in this document, we use only two grooves surrounding the central aperture. Influence of the number of grooves on the fluorescence directivity is thoroughly analyzed in the Supporting Information. The gold layer is 190 nm thick and is deposited on a glass coverslip substrate (refractive index \( n_g = 1.52 \)). A 60 nm thick chromium layer is deposited on top of the gold layer to further damp the transmission through the sample and confine the observation volume to the central aperture.\(^ {40}\)

Experimental measurements of fluorescence radiation patterns are performed with Alexa Fluor 647 molecules (A647, Invitrogen, Carlsbad, CA) and Rhodamine 6G (R6G, Aldrich, St Louis, MO). The molecules constantly diffuse in the central aperture and the half-space above the aperture. A647 and R6G are common fluorescent molecular probes with maximum absorption/emission peaks at 650/672 nm (respectively 525/550 nm), and a quantum yield of 30% (respectively 95%) in water solution. A water-based phosphate-buffered saline solution containing fluorescent molecules with micromolar concentration is deposited on top of the sample to ensure that on average 10 molecules are present in the aperture volume.\(^ {40}\) The excitation laser light is focused from below the sample (glass coverslip substrate) by a 1.2 NA microscope objective. The wavelength is set to 632.8 nm for A647 excitation or 488 nm for R6G, and the polarization is linear in both cases. To analyze the angular fluorescence emission, we record the fluorescence intensity distribution in the back focal plane (Fourier plane) of a 1.2 NA water immersion objective on a charge-coupled device (CCD) camera.\(^ {40}\) The radial coordinate in these images scales as the numerical aperture \( n \sin \theta \).\(^ {42}\) Throughout this study, we take \( n = 1.33 \) and express the emission polar angle \( \theta \) in the water medium, which is the most usual immersion fluid used for single molecule detection in solution.\(^ {4}\) The CCD images thus represent the intensity emitted for different angular directions \( \theta \) from the antenna, the polar graphs are referred to as radiation patterns. An essential point in this study is that all radiation pattern intensities are calculated back to average signal per molecule. We use fluorescence correlation spectroscopy (FCS) to quantify the average number of molecules \( N \) contributing to the overall fluorescence signal detected on the CCD camera. Full details on the FCS analysis can be found in ref 43 and in the Supporting Information of ref 40. The radiation pattern intensities are normalized by the actual number of molecules \( N \) measured for each experiment, providing the fluorescence count rate per molecule and the fluorescence intensity enhancement for each emission angle \( \theta \). All fluorescence intensities are integrated over spectral windows of \( \pm 20 \) nm around the central emission wavelength.

Our first set of results focus on the influence of the groove–nanoaperture distance \( a \) to control the 670 nm fluorescence emission of A647 molecules. From grating theory, it is known that coupling photons to surface waves requires momentum matching, which can be expressed as \( k_l \pm qk_g = k_{SP} \), where \( k_l = 2\pi n_g \sin \theta /\lambda \) is the far-field radiation parallel wave vector, \( q \) is an integer, \( k_g = 2\pi /P \) is the grating period, and \( k_{SP} = 2\pi /\lambda_{SP} \) is the wave vector of the surface wave, with \( n_g \) the real part of the surface wave refractive index. For infinite linear gratings, the momentum conservation law indicates that one should select \( \lambda = \lambda_{SP} / 2 \) to get maximum coupling along the normal direction (\( \theta = 0 \)), while \( \lambda = \lambda_{SP} / 2 \) provides minimum coupling toward the \( \theta = 0 \) direction. Here, we extend this formalism to circular gratings with the electromagnetic source directly located near the center of the grooves. To test the
influence of the groove—nanoaperture distance $a$, we first set a close to $\lambda_{SP} = \lambda/n_{SP}$ at the gold–glass interface. With this condition, we expect maximum emission beaming along the direction $\theta = 0$. The surface wave mode index is approximated by the surface plasmon dispersion relation at a flat gold–glass interface: $n_{SP} = (\varepsilon' \varepsilon_{g}/(\varepsilon' + \varepsilon_{g}))^{1/2}$, where $\varepsilon'$ denotes the real part of the metal permittivity and $\varepsilon_{g} = n_{g}^2$ is the substrate permittivity. This expression is only intended to serve as a guideline to select the parameter $a$, as the mode index $n_{SP}$ in the experiments is expected to depend on several parameters such as the groove period, number, depth, and shape. For the 670 nm emission of A647 molecules, we find $n_{SP} \approx 1.69$ and $\lambda_{SP} \approx 396$ nm. Experimentally, we set $a = 440$ nm as an approximation of the case for $a \approx \lambda_{SP}$. The second case of interest is for $a \approx \lambda_{SP}/2$ for which minimum emission along $\theta = 0$ is expected. This corresponds to taking $a = 220$ nm in our experiments.

Experimental radiation patterns with $a = 440$ nm $\approx \lambda_{SP}$ and $a = 220$ nm $\approx \lambda_{SP}/2$ are presented in Figure 2. For both cases, the groove period is the same at 440 nm. Back focal plane images display remarkable differences between the two cases; for $a \approx \lambda_{SP}$, a narrow beam with an extension of $\pm 14^\circ$ (half-width at half-maximum) emerges in the direction normal to the sample (Figure 2b), while for $a \approx \lambda_{SP}/2$, the fluorescence emission points toward $30^\circ$ with a minimum intensity in the direction normal to the sample (Figure 2c). Please note that the difference between the samples used for Figure 2b,c only concerns the groove—nanoaperture distances. The groove periods are identical. We relate the strong directionality to constructive or destructive interferences between the fluorescence light directly emitted from the central aperture into the far-field and the surface-wave coupled fluorescence emission that is reradiated by the grooves toward the far-field. The emission from the central aperture and the emission scattered by the surface grooves are in phase in the direction normal to the sample if the groove—aperture distance is a multiple of the wavelength $\lambda_{SP}$, and out-of-phase if $a$ is an odd multiple of half wavelengths. In the case of constructive interferences, we expect a peak intensity at $\theta = 0^\circ$, and an intensity drop for destructive interferences. This is exactly what is observed in Figure 2b,c. First-principle computations based on this physical optics picture are presented in the Supporting Information and correspond well with the experimental observations. The interference nature of the fluorescence beaming is further confirmed by recent studies on the optical transmission through plasmonic bull’s eye structures.

A nonintuitive aspect is that only a small fraction of the total emitted light is expected to be scattered by the surface grooves. However, at least three physical effects contribute to the observed fluorescence directionality. First, fluorescent molecules are nanoscale emitters whose radiation can be efficiently coupled to surface waves. Second, because the observed beaming is an interference process, the contributing waves may have different intensities while still leading to a significant contribution of the interference cross term (see physical optics calculations in the Supporting Information). Third, in the case where $a \approx \lambda_{SP}$, destructive interferences are found at $\theta = 23^\circ$ and $38^\circ$, which again contribute to narrowing the directionality along the main radiation lobe. Let us point out that the coherence length of the fluorescence emission (integrated over a 40 nm spectral window centered at 670 nm) is 11 nm, which is well above both the size of our antenna and the plasmon attenuation length for gold films.

Figure 2. Influence of the aperture–groove distance $a$ on the fluorescence radiation patterns and enhancement factors for Alexa Fluor 647 molecules. The different columns correspond to (a) a bare nanoaperture, (b) a nanoaperture surrounded by two grooves with $a = 440$ nm $\approx \lambda_{SP}$, (c) a nanoaperture with two grooves and $a = 220$ nm $\approx \lambda_{SP}/2$. The images represent the intensity distributions in the back focal plane of the 1.2 NA objective (Fourier images). The excitation power is 200 μW for all cases. Each image intensity is normalized by the average number of detected molecules calibrated by FCS and an intensity drop for destructive interferences. This is exactly what is observed in Figure 2b,c. First-principle computations based on this physical optics picture are presented in the Supporting Information and correspond well with the experimental observations. The interference nature of the fluorescence beaming is further confirmed by recent studies on the optical transmission through plasmonic bull’s eye structures.

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Figure 3. Photon-sorting capability of corrugated apertures for molecular fluorescence emission. The design used here has two grooves and a nanoaperture–groove distance \( a = 220 \) nm. (a) Sketch of the experiment; the central aperture is filled with a mixed solution of Alexa Fluor 647 and Rhodamine 6G. (b–d) Radiation patterns in the back focal plane of the objective for emission centered at 670 nm (b), 560 nm (c), and a combination of the two color images (d). (e,f) Fluorescence radiation patterns deduced from the images in (b,c).

The FCS calibration of the average number of detected molecules for each sample quantifies the radiation pattern intensity per molecule, and assesses the fluorescence enhancement along each emission direction (Figure 2d). The reference with the open solution is obtained from the known fluorescence enhancement for the nanoaperture with no groove.\(^{40}\) We quantify intensity enhancement factors of 80 for \( a \approx \lambda_{SP} \) and \( \theta = 0 \), and 32 for \( a \approx \lambda_{SP}/2 \) and \( \theta = 30^\circ \). The data in Figure 2d enables the evaluation of the directivity figure of merit for each antenna.

Following the classical IEEE definition,\(^{48}\) the directivity corresponds to the ratio of the radiated power density along the direction of strongest emission relative to the power density radiated by an ideal isotropic source emitting the same amount of total power.\(^{6,7}\) We experimentally quantify the antenna directivity by dividing the peak enhancement found in Figure 2d by the half of the average fluorescence enhancement integrated over the whole 1.2 collection NA (taking the half of the fluorescence enhancement in 1.2 NA accounts for the fact that the emission occurs only in the lower half-space, and no emission is radiated in the upper half-space).\(^{40}\) We measure directivities of 7.5 dB for \( a \approx \lambda_{SP} \) and 5.0 dB for \( a \approx \lambda_{SP}/2 \). These figures have to be compared to the 3.4 dB of a single aperture without grooves to demonstrate the benefit brought by the periodic grooves. A thorough characterization of the radiation patterns for increasing numbers of surface grooves is presented in the Supporting Information.

The interference picture to control the fluorescence directionality suggests that changing the emission wavelength strongly modifies the radiation pattern. To check this, we select the sample with a groove–aperture distance \( a = 220 \) nm. This corresponds to \( a \approx \lambda_{SP}/1.8 \) for Alexa Fluor 647 emission centered at 670 nm, but is amounts to \( a \approx \lambda_{SP}/1.4 \) for Rhodamine 6G emission centered at 560 nm. Figure 3 summarizes the experimental results. As expected from Figure 2c, the A647 emission at 670 nm is directed toward 30°, while the R6G emission at 560 nm is nicely centered along \( \theta = 0 \) with a HWHM extension of 22°. These results clearly demonstrate photon sorting along different directions for spectrally different molecules in solution. The emission directionality follows the behavior expected from transmission experiments through corrugated slits; for short wavelengths, the transmitted light is beamed along the optical axis, while for longer wavelengths the transmitted light is radiated off-axis.\(^{24,37}\) However, this is the very first time such behavior is reported for individual quantum emitters randomly located directly inside the antenna. This system is not simply a circular grating, the driving source (feed element) is directly incorporated into the antenna device. This system allows sampling for a few molecules in a highly concentrated solution and strongly increases the emission intensity and directivity. The total size of this device is 1.5 \( \mu m \), which is comparable to the axial dimension of Yagi–Uda antennas,\(^{20}\) and realizes a significant reduction compared to the 4.5 \( \mu m \) size of our previous demonstration.\(^{40}\)

The azimuthal symmetry of A647 emission in the case of Figure 3 does not realize the optimum photon directional concentration. One would like all the red photons to be emitted along a given direction, and simultaneously all the green photons to be beamed along a different angle. To achieve this, the circular symmetry of the antenna needs to be broken. A first method would be to mill asymmetric split grooves with different periods and groove–aperture distance on the left/right of the aperture. A more elegant way to break the antenna symmetry is to shift the
aperture position by a distance $\delta$ relative to the grooves center. For the experiments presented in Figure 4a, the first groove radius is 440 nm (which means $a = 440$ nm if $\delta = 0$). For the radiation patterns shown in Figure 4b–f, we set $\delta = +150$ nm (data corresponding to $\delta = +66$ nm are presented in the Supporting Information). With this set of parameters, both the red and green emission are beamed toward angular directions of $-14$ and $-23^\circ$ with HWHM extensions of 14 and 28$^\circ$, respectively. The main radiation lobe points always in the direction opposite to the aperture displacement, as expected from physical optics calculations (see Supporting Information). Moreover, for the range of displacements studied here we observe a linear relationship between $\delta$ and the main radiation direction for both emission wavelengths (see the inset in Figure 4e). Remarkably, this experiment demonstrates directional emission of fluorescence over a broad spectral window ranging from 540 up to 690 nm. The fluorescence intensity enhancement (per molecule) is presented in Figure 4f. As compared to the symmetric case $\delta = 0$, the intensity enhancement is lower as the aperture is shifted from the center, yet a significant enhancement is still found. The aperture shift can be compared to an impedance mismatch, leading to a lower excitation intensity inside the aperture (receiving mode) and to a lower emission rate enhancement (transmitting mode). We also note the fluorescence count rate enhancement is much lower for R6G than for A647. This is a direct consequence of (i) the lower intensity enhancement for 488 nm excitation than for 633 nm due to the poor plasmonic properties of gold below 500 nm, and (ii) the high quantum yield of R6G in water solution. Similar effects have already been observed for noncorrugated apertures.45 Concerning the antenna directivity figure of merit, the directivities when $\delta = 150$ nm are 6.5 dB for $\lambda_R = 670$ nm and 4.4 dB for $\lambda_V = 560$ nm, which are approximately 1 dB less than the directivities found for $\delta = 0$.

In conclusion, this work opens new nanoantenna design paths to tune the fluorescence emission directivity for spectrally different quantum emitters with random position and orientation inside a sensing unit. We clearly demonstrate that the fluorescence beaming results from an interference phenomenon. The main control over the fluorescence directionality stems from the phase relationship between the emission directly radiated from the nanoaperture and the surface waves mediated emission that is scattered by the grooves. Extending these concepts to the near-infrared spectral range where surface plasmon resonances become sharper would yield narrower angular divergence and
higher antenna directivities. The concepts developed here suggest milling the corrugated aperture antennas in aluminum to extend the spectral range over the 400—600 nm window commonly used for fluorescence sensing. Directivities and fluorescence enhancement factors are not expected to be as good as for gold, yet intensity enhancement of 40-fold and angular divergence of ±20° appear experimentally feasible for aluminum antennas that could be designed for any wavelength in the visible domain. Corrugated nanoaperture antennas thus offer a high degree of tunability and can operate over a large spectral range, which makes them essential devices in the context of nanophotonics. The compatibility with single molecule analysis in highly concentrated solutions suggests novel biophotonic applications, such as multicolor directional enhanced fluorescence sensing of several molecular probes.

After the submission of our manuscript, another group also demonstrated fluorescence beaming with a corrugated aperture antenna, and the findings are consistent with our results shown in Figure 2.

## ASSOCIATED CONTENT

Supporting Information. Influence of the number of grooves, first-principle physical optics model, finite difference time-domain simulations, and influence of the aperture lateral displacement. This material is available free of charge via the Internet at http://pubs.acs.org.

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