

Supplemental material to:

Photoactivated capture molecule immobilization in plasmonic nanoapertures in the ultraviolet

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Experimental

Substrate cleaning. The substrates (nanoaperture arrays/glass) were cleaned using solvent wash. The wash included acetone, isopropyl alcohol and methanol. After solvent wash, samples were rinsed with doubly distilled water (ddH₂O) and dried using nitrogen. This was followed by oxygen plasma cleaning using a Harrick plasma cleaner. The plasma cleaner was operated at the medium power setting (200 W) for 5 minutes. At this point, the samples were placed in a closed petri dish and set aside for 30 minutes before any further processing.

Passivation. Briefly, placing the substrates in 1 mM solution of n-octadecyl phosphonic acid in methanol for 48 hours forms the passivating layer. Substrates were cleaned in methanol and dried under nitrogen. After passivation, samples were annealed under nitrogen for 4 hrs at 90° C. The physisorbed phosphonic acid was removed using triple methanol and ddH₂O washes.

Silanization. After cleaning, the substrates were placed in a Fisher Scientific oven at 135° C with a small vial containing 1.5 ml of APTES. The oven was sealed, pumped down, and purged 3 times with ultrapure nitrogen. After 8 hours, the oven was purged with nitrogen and the substrates were removed.

Immobilization of ATFB. The N-hydroxysuccinimide (NHS) ester group of the ATFB molecule reacts with the amine group on the silane to produce a stable amide bond and N-hydroxysuccinimide (NHS) is released as a byproduct. NHS esters react with deprotonated primary amines at neutral to basic pH values. A stock solution of ATFB is made in DMSO at 10 mg/mL. 50 µL of this solution was used to form an ATFB layer on amino-silanized substrates by carrying out the reaction in 1 mL of 100 mM bicarbonate buffer at pH 8.5. The reaction was carried out at 4° C for 1 hour with constant shaking.

UV photoactivation. Probe biotinylated oligonucleotide (22-mer) of 20 µM and 200 µM were used for this study. The stock solutions were prepared in 100 mM phosphate buffer, pH 7.4. 2 µL of biotinylated oligonucleotide was pipetted onto the substrates, which were exposed to UV light for the desired period of time (0 to 3 minutes). Substrates were washed in 3X SSC buffer containing 1 mM EDTA and 0.1% SDS.

Hybridization. Stock solution of 50 nM target sequences, complimentary to the immobilized probe sequences, were made in 3X SSC buffer containing 1 mM EDTA and 0.1% SDS. The target sequences are either radio-labeled with P-32 or fluorescently labeled during synthesis with Cy-5 dye. For radio labeling, target oligonucleotides were 5'-end-labeled with [γ -³²P] dATP using the T4 polynucleotide kinase labeling kit (New England Biolabs). The reaction mixture consisted of 10 pmol of oligonucleotide, 3 µl of 10X T4 polynucleotide kinase buffer, 20 µl of T4 polynucleotide kinase, 5 µl of [γ -³²P] dATP (6000 Ci/ mmol, perkin elmer) and ddH₂O to a final volume of 50 µl. The mixture was incu-

bated at 37° C for 30 min. 10 μ l of 0.2 M EDTA (pH 8.0) was added to terminate the reaction. The product was purified using sephadex G-25 columns. The solution was dried using a speed vac. Dried oligonucleotide was re-suspended in 3X SSC buffer containing 1 mM EDTA and 0.1% SDS. Hybridization reactions were carried out at 40° C for four hours. Samples were scanned after washing them in 3X SSC buffer containing 1 mM EDTA and 0.1% SDS. In case of radio labeled sequences, imaging was performed by using phosphor screens.

Electromagnetic enhancement in aluminum nanoapertures

Methods. Electromagnetic calculations were performed using COMSOL multiphysics v3.5a; a glass substrate is assumed, on top of which a 100 nm thickness Al film is placed; the upper region is air or water, as appropriate for experimental conditions. The dielectric properties of Al were incorporated via the complex dielectric constant as measured by spectroscopic ellipsometry from 300-1600 nm. The size of the computational space was set by the nanoaperture array periodicity with periodic boundary conditions applied on the faces. Nanoaperture arrays were excited by linearly polarized light and enhancements were calculated for two directions of incidence (air side, substrate side). Intensity enhancement was calculated by integrating the total field intensity within the volume of a 10 nm slice at the bottom of the nanoaperture and dividing by the total integrated intensity within the same volume in the absence of the Al film.

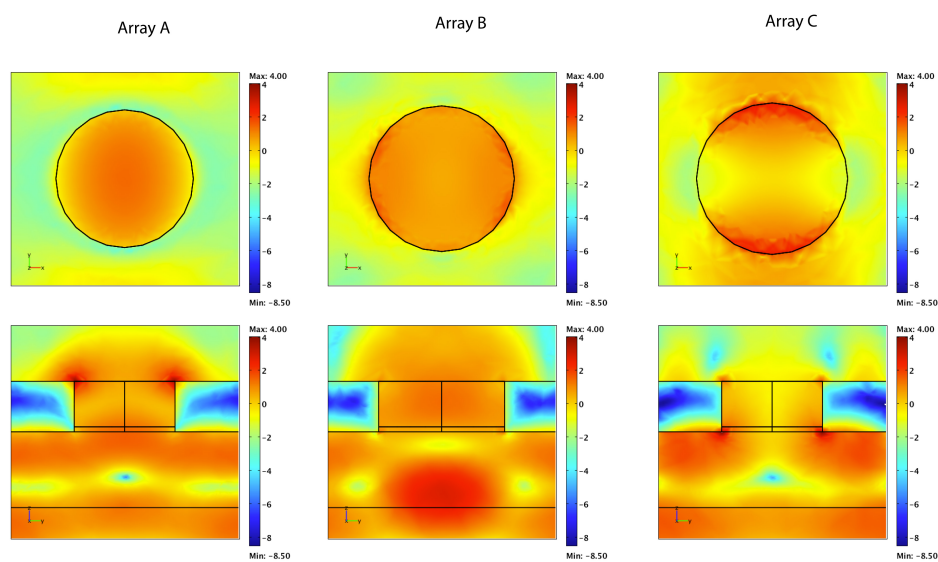


Figure S1. *Cross-section intensity distributions obtained from electromagnetic calculations for the three different nanoaperture array parameters. Intensity is on a log scale.*

UV enhancement. The first set of calculations were performed to assess plasmonic excitation enhancement during UV exposure. According to experimental conditions, light at 365 nm wavelength was incident on the nanoaperture array through the substrate. The upper region was water. Corresponding to the three different experimental nanoaperture geometries, Figure S1 shows the calculated intensity distribution along the bottom of the nanoapertures and through their cross-sections. In all cases, relatively uniform illumination is obtained near the glass surface, while localized surface plasmon resonances can be observed at the top and bottom nanoaperture interfaces in the cross-section images.

The UV exposure enhancement is calculated by averaging the intensity within the 10 nm tall volume near the glass interface where the ATFB molecule is bound. A plot of this enhancement versus wave-

length is shown in Figure S2 for array C. At 365 nm, the exposure enhancement factor is 3.5, but the peak in the short wavelength response is near 390 nm. Redesign of the nanoaperture array parameters can shift this peak to 365 nm.

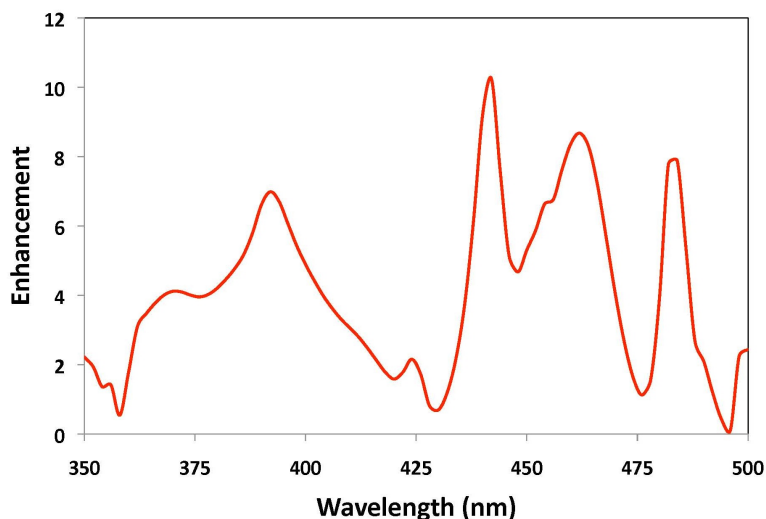


Figure S2. *Intensity enhancement factor within a nanoaperture obtained from electromagnetic calculations for array C (500 nm pitch, 200 nm aperture diameter) under conditions for UV activation.*

Fluorescence enhancement. Additional calculations were performed to illustrate the fluorescence enhancement measured for the Cy-5 dye. According to fluorescence measurements made with the microarray scanner, incident light was from the top side, which is now air, at 635 nm wavelength. Calculation of the intensity enhancement within the nanoapertures under these conditions is shown in Figure S3. Again, the peak enhancement is at a different wavelength than used for excitation, demonstrating that further design is necessary in order to simultaneously maximize enhancement at the two wavelengths of interest, and under different experimental conditions.

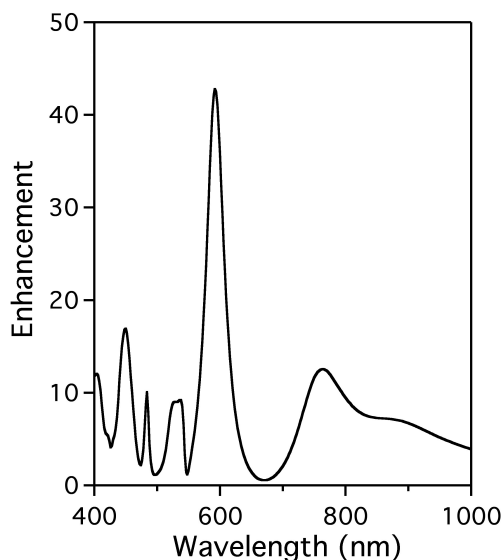


Figure S3. *Intensity enhancement factor within a nanoaperture obtained from electromagnetic calculations for array C under conditions appropriate for fluorescence measurements.*

Enhancement of the excitation light alone is not sufficient to account for the total fluorescence enhancement obtained for Cy-5 (or any other fluorophore, for that matter). Because the dye is confined within a sub-wavelength structure, the influence of the electromagnetic environment on the emission of

the dye must also be considered. More detail about this aspect is provided in references [31] and [39], but briefly, under weak excitation conditions, enhancement in the emission process is via the change in quantum efficiency of the dye, which is based upon modification of the radiative and non-radiative transitions rates by the nanoaperture. Under high excitation conditions when the emission of the dye saturates (i.e. excitation rate approaches transition rate), only the change in radiative transition rate contributes to fluorescence enhancement.