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Plasmonic Nanostructure Functionalization for Surface Enhanced Fluorescence Bio-Detection †

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Abstract: Plasmonic nanostructures represent a suitable platform for the detection of biomolecule interactions. Their surface functionalization can be performed through different strategies. Optimal thickness, homogeneity, and hydrophilicity of the functional layer can play a crucial role in defining the sensing capabilities required to perform bioassays. In this framework, a combination of tetraethylorthosilicate (TEOS) and a commercial polymer (MCP) was evaluated to improve these features. In our more recent studies, we focused on plasmon enhanced fluorescence for the detection of a microbial-derived synthetic oligonucleotide. An effective improvement of the fluorescence signal was detected for the combined TEOS and MCP coating.

Keywords: nanostructured surface functionalization; multilayered nanocoating; plasmon enhanced fluorescence

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1. Introduction

Biosensors represent a class of promising analytical devices where physical phenomena are coupled with biological elements to enable the acquisition of information inherent in chemical processes. Underlying the definition, biosensors are a wide class of devices characterized by different sensitivity, robustness, and area of application. In particular, sensors based on surface plasmon resonance (SPR) are especially convenient in terms of application spectrum, as these platforms can be adapted for many different types of analytes [1].

The feasibility of shaping the surface chemistry by different strategies enables the development of interfaces with specific characteristics, such as thickness, homogeneity, and hydrophilicity, which can crucially be affecting the sensing capabilities required to perform bioassays. In this framework, one of the more fascinating fields of application is represented by plasmonic enhanced fluorescence (PEF). PEF occurs when plasmons can be coupled with the fluorescence features of specific fluorophores, changing their signals. It is a well-described effect, depending on several elements, but primarily the dependency between the fluorophore and the metal distance, the increasing of the excitation field and the modulation in photon emission mediated by the metal [2].

From an implementation perspective, there is an unexpressed potential for a wide range of methodologies that are routinely performed in many laboratories, affected by a low sensitivity and reliability underlying the properties of some common fluorophores [2].

In this view, starting from our nanoplasmonic grating (NPG) [3] we build up a convenient approach to make it suitable for PEF application. First, the plasmonic nanostructure was covered with a thin layer of tetraethyl orthosilicate (TEOS) [4] which improves the chemical homogeneity of the surface. Then a commercial Lucidant MCP polymer [5] was coated on the TEOS layer. The polymer decreases the contact angle of the surface preventing the aggregation of biomolecules during immobilization. Since the overall thickness of the functionalization bi-layer is in the order of 10 nm, we had the advantage to keep the fluorophore close to the sensing surface, as crucial for exploiting the PEF modality of detection. To verify the capabilities in PEF detection, we resorted to a DNA microarray, where, in building up the assay, the detector probe was labeled with the dye Alexa Fluor 750 (AF750) selected thanks to the spectral overlap with the plasmonic features of our grating. Several surfaces were examined and the corresponding images compared by measuring the fluorescence intensity by means of a microarray scanner, in the so-called front-front configuration (Figure 1).

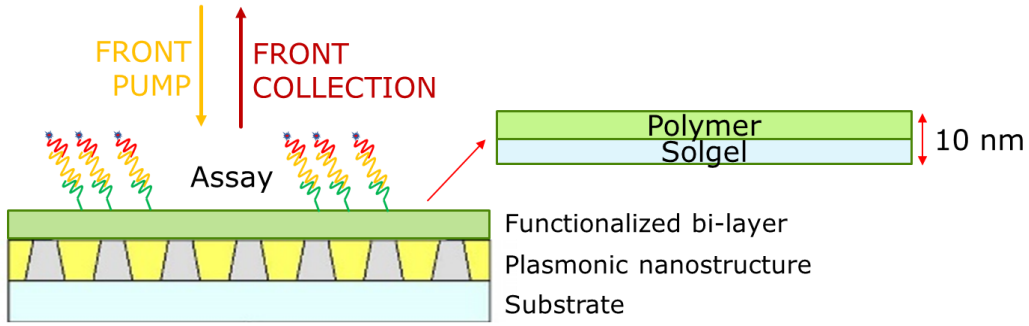

Figure 1. Sketch of the functionalized plasmonic nanostructure and measurement scheme.

2. Materials and Methods

2.1. Nanoplasmonic Grating Fabrication

Standard NPGs are produced by following an established fabrication protocol based on colloidal lithography. A monolayer of colloidal nanoparticles, acting as a lithographic mask, is deposited on top of a dielectric layer of a few hundred nanometers, followed by a step of dry etching that produces an ordered pattern of nanospheres on top of pillars at well-defined distances and geometries. After this etching step, the substrate is coated with a thin gold film with controlled thickness, and the colloidal mask is removed through a lift-off step creating a final hexagonal lattice crystal geometry [3].

2.2. Functionalized Bi-Layer

Sol-gel preparation was previously established using tetraethoxysilane (TEOS) as a silica monomer [4]. The sol precursor is prepared by mixing TEOS (8 mL), ethanol (EtOH) (20 mL), and water (H_2O) (3.75 mL) under stirring at room temperature (RT) for 24 h. Subsequently, combining EtOH and methyl cyanide (MeCN) a 6 mL volume of EtOH/MeCN (*v*/*v* = 1:1), and chloride acid (HCl) (up to pH 4) is added to 9 mL of the sol. The mixture was then maintained under stirring at 50 °C for 1 h. The resulting TEOS solution is gently mixed in a closed vessel at RT. Before the coating process, both the flat gold and nanoplasmonic substrates were extensively cleaned. The sol-gel films are then deposited by dip coating using an automatized device setting the withdrawal speed of 20 mm/s. After the deposition, all the films were dried at RT for 48 h [4].

Lucidant MCP polymers, as well as TEOS coating, are consolidated systems employed in our laboratories (ref). The polymer is coated by drop-casting on the surfaces and after 30 min, it is roughly washed in water. Follows a curing step at 80 °C for 15 min, after which each platform (NPG, flat goldand glass) is ready for biomolecule immobilization.

2.3. Assay

The assay for the detection of DNA was designed in a three-component format.

A capture oligonucleotide is printed on derivatized substrates to form an array using a piezoelectric spotter (SciFLEXARRAYER S3; Scienion, Germany). After an overnight incubation, all residual reactive groups of the coating polymer were blocked and then the surfaces were rinsed with a warmed buffer (50 °C) as pre-condition step before the DNA hybridization.

The target oligonucleotide designed on E.coli bacteria [7] is then diluted to 250 nM in a total volume of 4 µL in the hybridization buffer (SSC 2X, Saline Sodium Citrate 2X + SDS 0.1% + BSA 0.2 mg/mL) and immediately applied to microarrays. The surfaces are transferred to a humidified incubator to allow the DNA hybridization at a temperature of 65 °C for 2 h. Since the target sequence is now present on the microarray, the oligonucleotide detector labeled with Alexa Fluor 750 dye (AF750) [7] at 1 µM can be finally applied on the surface, and similarly as the previous molecule is hybridized for 2 h at 40 °C. The surfaces were then again extensively washed with SSC buffer at different ionic strength (SSC 4X + SDS 0.1%, two times; SSC 0.2X + SDS 0.1%, two times; SSC 0.1X + SDS 0.1%, two times) and dried to be measured.

2.4. Optical Measurement

The different functionalized platforms were designed to be analyzed on a commercial microarray scanner. This gave us the advantage of characterizing the fluorescence signal with a quick, simple, user-accessible, and robust method.

The fluorescence images were collected resorting to an InnoScan 710-IR (Innopsys) [6]. The fluorophore AF750 linked to a detector oligonucleotide was excited through a laser at 785 nm at low laser power (5 mW) and the collection was performed at 1% of PMT. From the resulting scans, regions of interest (ROIs) were defined matching the spots grid and the total fluorescence intensity for each ROI generated by subtracting the background signal.

3. Results and Discussion

Four samples have been fabricated resorting to the same techniques, their characteristics are reported in Table 1 and corresponding fluorescence images are reported in Figure 2.

Table 1. Summary of the sample treatments.

Sample	Nanostructured	TEOS	MCP	Assay
Glass MCP	No	No	Yes	Yes
Flat gold TEOS MCP	No	Yes	Yes	Yes
NPG MCP	Yes	No	Yes	Yes
NPG TEOS MCP	Yes	Yes	Yes	Yes

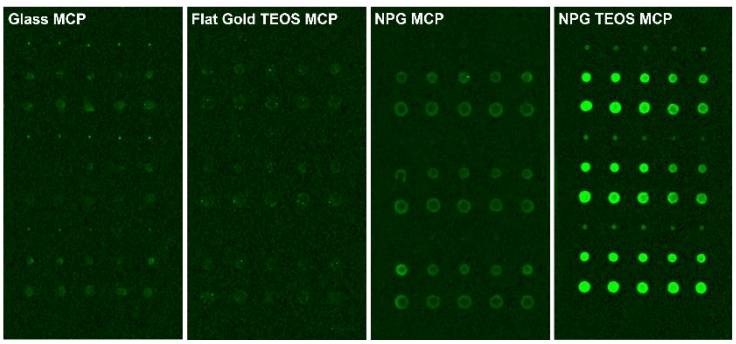

Figure 2. Fluorescence emission images were collected with Innopsys microarray scanner.

The images reported in Figure 2 show the compatibility of our platform with PEF detection in imaging mode resorting to a benchmark instrument.

It is evident that the fluorescence intensity is higher (perceived as brighter) on NPG with the combination of the bi-layer TEOS + MCP with respect to all the other platforms.

Since the ratio of oligonucleotides hybridization is 1 to 1, the amount of drops of a single spot is directly correlated to the number of fluorophores present on that region of the surface. Thus, starting from the total intensity values given by the microarray scanner, we calculated for each platform an average of the spots with the same number of drops (Figure 3). This result resembles the observations given by the imaging scanner but also reveals a different behaviour in the signals between non-nanostructured and nanostructured surfaces.

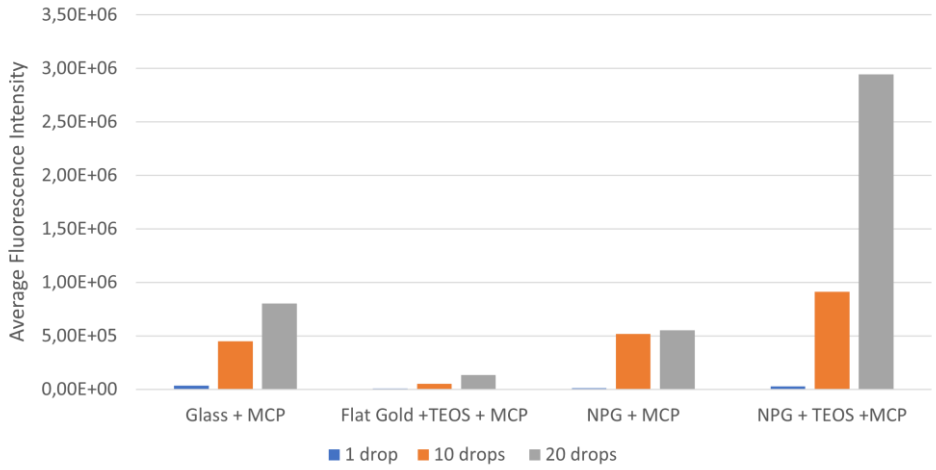

Figure 3. Fluorescence intensity average collected with Innopsys microarray scanner.

For this reason, in a final analysis we decided to plot a trend of each sample in terms of average fluorescence intensities versus the number of drops (Figure 4). While it is shown a linear or semi-linear trend in most of the surfaces, in the case of the NPG TEOS + MCP it should be noticed a supralinear behaviour. In any case, it is evident the role of the bi-layer in improving the fluorescence intensity.

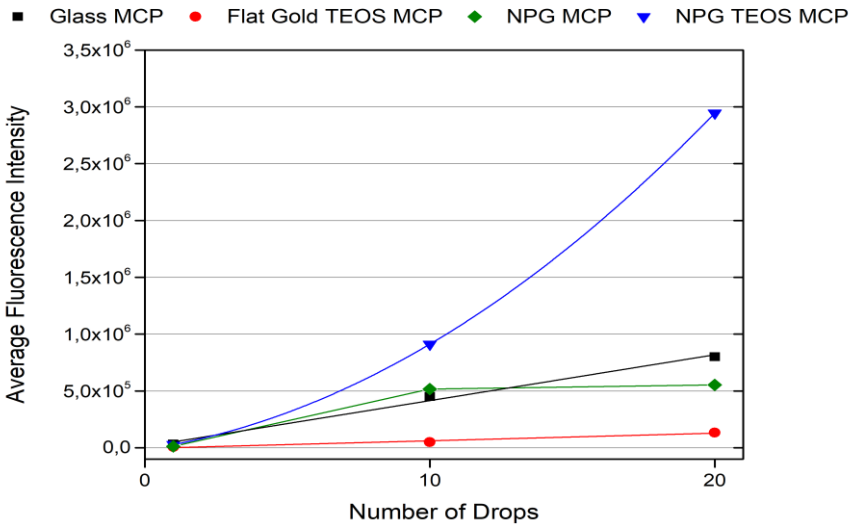

Figure 4. Fluorescence intensity variation trends in function of the number of drops.

4. Conclusions

Enhancing a fluorescence signal resorting to a plasmonic platform may be complex and many parameters can be involved. Despite this, in the present work we presented a well-established protocol to place a fluorophore into an optimal configuration to exploiting part of this phenomenon. Our nanostructured plasmonic surfaces have also been shown to perform properly for this purpose, so further studies will be conducted to corroborate our achievements.

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