

Microstructured molecular BIO-gratings by means of UV induced denaturation - INVITED

Augusto Juste-Dolz¹, Martina Delgado-Pinar^{2,*}, Miquel Avella-Oliver^{1,3}, Estrella Fernández¹, Jose Luis Cruz², Miguel V. Andrés², and Ángel Maquieira^{1,3}

¹Instituto Interuniversitario de Investigación de Reconocimiento Molecular y Desarrollo Tecnológico (IDM), Universitat Politècnica de València, Universitat de València, 46022 Valencia, Spain

²Laboratory of Fiber Optics - Institut de Ciència dels Materials (ICMUV), Universitat de València, Catedrático José Beltrán 2, 46980 Paterna, Valencia, Spain

³Departament de Química, Universitat Politècnica de València, 46022 Valencia, Spain.

Abstract. Rapid, reliable and low cost techniques to fabricate biosensors is a hot topic nowadays. Here, we present a BIO-grating fabricated by means of local, selective denaturing of molecules using UV radiation. A phase-mask is used to generate an interferometric pattern of 1420 nm pitch that, when illuminating a biolayer of BSA molecules lead to its periodic deactivation. After the biorecognition of the specific antibody, aBSA, a BIO-grating is generated due to the height difference between the protein, and the complex protein + antibody. We present the optimization of the fabrication of the BIO-gratings and their AFM characterization. Also, the biosensor performance in terms of limit of detection and limit of quantification will be presented.

1 Introduction

There exists a number of biomedical applications that exploit patterned biomacromolecules such as organ-on-a-chip, drug delivery or biosensing [1-3]. The development of fast, low-cost and reliable techniques to fabricate such microstructures of molecules has a potential impact on industry and diagnosis. Among the existing methods we can mention the deposition of biolayers on already patterned substrates by means of laser interference or photolithography [3, 4], for example. Also, there are strategies where a pattern of molecules is stamped on a uniform substrate, such as microcontact printing [5]. When these techniques are used to produce biosensors, the sensitive biolayer already shows some topography that determines the zero-response of the device.

Here, we present a technique to fabricate diffractive molecular gratings on planar surfaces by means of a mild denaturation of a uniform biolayer of molecules in a periodic manner. An interferometric pattern produced by a phase-mask illuminated by UV radiation (in a similar way that the method widely employed for the photoinscription of Bragg gratings) will be used. The UV radiation locally deactivates the molecules that form the uniform biolayer [6], according to the maxima and minima of the interferometric light pattern. This deactivation induces a patterning in the functional activity of the molecules while it does not involve a topographic change in the biolayer, since the UV fluence illuminating the biolayer does not reach the ablation level. This minimizes the zero-response of the biosensor, and together with the uniformity of the structures and the

repeatability of the method when compared to microcontact printing, improves the performance of the biosensor.

2 Fabrication of the BIO-gratings

The complex protein-antibody system used for optimizing the fabrication parameters was a well-established model: bovine serum albumin (BSA) – antiBSA (aBSA), from Sigma-Aldrich (Madrid, Spain). The first step was to determine the adequate concentration of BSA in sodium phosphate buffer (PBS) to deposit uniform biolayers of proteins onto silica substrates (microscope slides, in our case), which resulted to be 20 µg/mL.

The biolayers were then illuminated with UV-radiation to locally deactivate the functionality of the molecules. Once the proteins are exposed to the sample containing the antibody (aBSA), only those which were not exposed to UV radiation, that is, the active ones, bind to the antibody. The result is a diffractive BIO-grating that plays the role of the transducer of the biosensor. Figure 1 shows a scheme of the fabrication process and the resultant BIO-grating after the immunoassay.

The UV-light interferometric pattern is generated by a 244 nm, Ar-doubled laser illuminating a phase mask (period: 1420 nm). The biolayer is placed next to the phase-mask. The power of the laser, as well as the sweeping velocity of the beam along the phase-mask can be controlled independently. Both together determine the fluence over the BSA biolayer, which is the parameter that needs to be optimized. We found that it was necessary to achieve a compromise between both parameters: same

* Corresponding author: martina.delgado@uv.es

fluences obtained with different illumination conditions lead to different diffraction efficiencies of the BIO-grating.

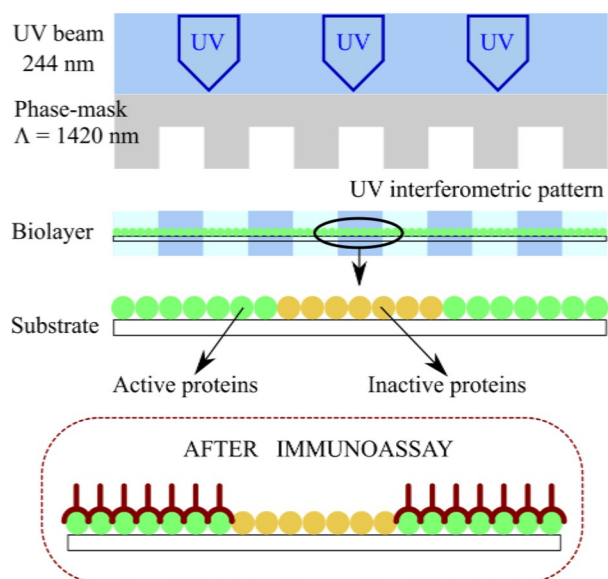


Fig. 1. Scheme illustrating the fabrication process and the resultant BIO-grating before and after the immunoassay.

It is worth to note that after the biolayer is UV-illuminated, there is not a topographic change in the BSA molecules yet, see Fig. 2, which shows an AFM image of an irradiated BSA biolayer and the correspondent height profile. Only after the immunoassay, when the specific protein and target bind together, the BIO-grating is in operation. This feature makes this type of biosensors especially robust against non-specific bindings, and enhances the detection limit (DL) of the biosensor since it reduces dramatically its zero-response, hence the signal to noise ratio of the diffracted signal.

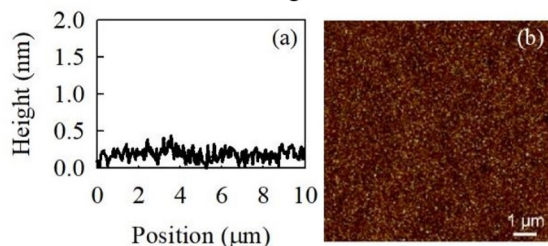


Fig. 2. (a) Height profile and (b) AFM image of a BSA biolayer after UV-irradiation. UV-fluence: 2.5 J/cm^2 , laser power: 55 mW, sweeping velocity: 2.2 mm/s .

We fabricated a series of BIO-gratings under different illumination conditions that were tested using dilutions of $10 \mu\text{g/mL}$ of aBSA in PBS buffer. The gratings were interrogated using a 532 nm CW laser, and the diffraction efficiency was determined as the ratio between the power of the first and the zeroth order diffracted beams. Figure 3 (a) shows the results: three different curves, corresponding to laser powers of 27.5, 55 and 100 mW are shown. For each curve, the fluence was controlled by changing the sweeping speed of the beam along the biolayer. From our results, the maximum diffraction efficiency is obtained at medium laser powers and a fluence of 2.5 J/cm^2 : for these illumination conditions, the maximum height modulation in the BIO-grating is obtained, while its duty-cycle remains 50%,

approximately. For lower powers and low fluences, the threshold to deactivate the proteins might not be achieved, while in the case of 100 mW laser power, too many BSA molecules are deactivated, leading to a duty-cycle lower than 50%; these features can be observed in Fig. 3 (b).

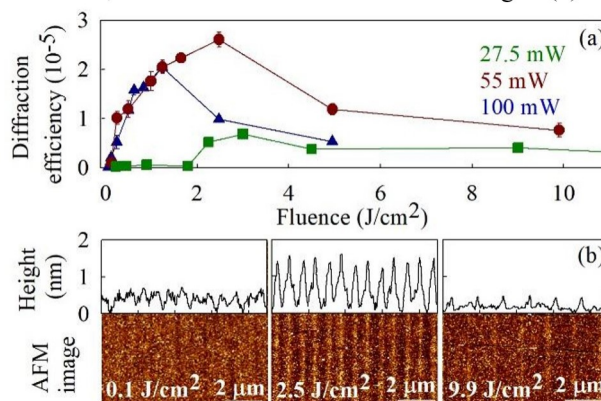


Fig. 3. (a) Diffraction efficiency of the BIO-gratings as a function of UV fluence after the immunoassay. (b) Height profile (upper) and AFM image of three BIO-gratings, for 55 mW UV-power.

Complete immunoassays were performed for the optimized BIO-gratings in PBS buffer dilutions of different concentrations of aBSA, as well as in human serum dilutions. The DL and quantification limits of the biosensors are 36 ng/mL and 100 ng/mL (aBSA in human serum), and 53 ng/mL and 164 ng/mL (aBSA in PBS buffer) [7]. It is also worth to note that the BIO-gratings remained active for at least 30 days stored at 4°C .

3 Conclusions

We have presented a reliable, rapid fabrication method of BIO-gratings for biosensing, with enhanced performance compared to other techniques, for example microcontact printing, as it is detailed in [7].

References

1. G. Arrabito, V. Ferrara, A. Bonasera, B. Pignataro, *Small* **16**, art. 1907691 (2020).
2. S. Qiu, J. Ji, W. Sun, J. Pei, J. He, Y. Li, J. J. Li, G. Wang, G., *Smart Mater. Med.*, **2**, 65–73 (2021).
3. M. I. Lucío *et al.*, *Biosens. Bioelectron.*, **193**, art. 113561 (2021).
4. S. Fruncillo, X. Su, H. Liu, L. S. Wong, *ACS Sens*, **6**, 2002 (2021).
5. A. Perl, D. N. Reinhoudt, J. Huskens, *Adv. Mater.* **21**, 2257–2268 (2009).
6. W. F. Heinz, W. F., *Lab Chip* **11**, 3336–3346 (2011).
7. A. Juste-Dolz *et al.*, *ACS App. Mat. & Interfaces*, **14**, 41640–41648 (2022).

Funding: MCIN/AEI/10.13039/501100011033 (grants: PID2019-110713RB-I00, TED2021-130200B-I00 and PDI2019-104276RB-I00).