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Real-time Biosensing of Proteins on a DVD Nanoplasmonic Grating

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ABSTRACT

Development of label-free, highly sensitive, miniaturized surface plasmon resonance sensors enables real-time quantification of biomolecule interactions at atomic-levels, desirable for medical diagnostics and which will allow rapid clinical decisions. However, multi-target diagnostic assays require skilled labor, expensive materials, lengthy manual steps, as well as complicated analysis steps. Here, we develop a microfluidic-integrated digital optical disc (DVD) grating as a metasurface, which is coated with titanium-silver-gold (Ti-Ag-Au, 10, 30, and 15nm) for real-time monitoring of biomolecular interactions and binding affinities. Device fabrication process consists of poly (methyl methacrylate) (PMMA) microfluidic channel assembly on nanoplasmonic DVD surface gratings *via* double side adhesive (DSA) layers. Compared with other nano- and micro-fabrication methods, DVD-based sensor fabrication is relatively simple, cost-effective, and enables large-scale fabrication with minimum efforts. The plasmonic microfluidic chip surface was illuminated with a broadband light source and the normal reflection signal was monitored using a customized optical-setup. Maximum bulk sensitivity (337 nm/RIU) was observed with 30 seconds of etching period and low glycerol concentration (5%, v/v). Red-shifts of peak-wavelength (~16 nm) upon glycerol concentrations were observed as a function of time (seconds). A 0.6 nm peak-wavelength shift was observed in the step of EDC/NHS coupling and continuous protein A/G and G binding resulted in 0.353 ± 0.211 nm and 0.667 ± 0.116 nm ($n=3$, $p>0.05$). The presented platform could be potentially applicable to detect and real-time monitor of various biotargets including bacteria, cells, viruses, and proteins.

Keywords: Digital optical disc (DVD), Metasurface, Surface plasmon resonance, Biosensors

1. INTRODUCTION

Surface Plasmon Resonance (SPR) sensors are based on trapped electromagnetic waves at metal-dielectric interfaces and show high sensitivity and quick response, enabling applications in medical diagnostics, food safety, biosafety and environmental monitoring [1-3]. Due to light controlling capability and intrinsic losses of metasurface, metasurface-based plasmonic sensors present enhanced sensitivity [4]. The sensing response is based on the interaction and momentum transfer of p-polarized light on a metal-dielectric interface at certain angle (SPR angle) to match incident and metal-free electrons momentum, and therefore, surface plasmonic wave (SPW) propagate along the metal-dielectric interface [2, 5]. Refractive index (RI) sensing of plasmonic metasurface is principally based on resonance wavelength shifting due to the alteration of propagating mode and coupling conditions between incident light and SPW [5]. Further,

engineered metasurface shows asymmetrical plasmonic resonance (also known as Fano resonance), which is caused by the hybridization of plasmonic mode due to the interference of plasmonic particles [6]. Plasmonic metasurface-based sensors are fabricated with different material layers, such as metal-dielectric-metal, metal-dielectric, and all metal configurations [4, 7]. For example, all metal plasmonic sensors were reported a sensitivity of 400 nm/RIU for RI variations from 1.312 to 1.352 [8]. In general, plasmonic metasurface-based sensors have been however fabricated with expensive and time-consuming methods (*e.g.*, electron-beam and photolithography) [1, 9]. Even though there have been proof-of-concept studies conducted, there is still a very limited number of studies performed for on building low-cost plasmonic sensors using optical discs surfaces [10, 11].

Here, we demonstrate a cost-effective microfluidic-integrated DVD grating as a metasurface biosensor to monitor protein bindings in real-time. Device fabrication is facile, including the assembly of poly (methyl methacrylate) (PMMA) onto a DVD surface *via* a double side adhesive (DSA) layer. Maximum bulk sensitivity (337 nm/RIU) was observed with 30 seconds of etching period at low glycerol concentration (5% v/v). Approximately 0.6, 0.667 ± 0.116 , and 0.353 ± 0.211 nm nm peak-wavelength shifts were observed for EDC/NHS, protein G and A/G binding, respectively.

2. MATERIALS AND METHODS

Chemicals and Materials: 11-Mercaptoundecanoic acid (11-MUA), N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), and N-Hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich (St. Louis, MO). Protein G and A/G were purchased from Fisher Scientific (Hampton, NH). PMMA from MacMaster Carr, (Elmhurst, IL), and DSA from iTapestore, (Scotch Plains, NJ) were purchased to fabricate microfluidic channels.

Methods: The schematic depicts the proposed nanoplasmonic DVD sensor (Figure 1a). DVD surface grating was cleaned with ethanol and methanol mixture (1:1) at 65°C for 5 minutes to remove existing coatings. The surfaces were then washed with deionized (DI) water and dried with filtered compressed air. Finally, the DVD surfaces were coated with Ti (10 nm), Ag (30 nm), and Au (15 nm) through an evaporation process to generate the plasmonic layers. The coated surface were re-cleaned with ethanol and methanol mixture, and washed with DI and dried with the filtered compressed air to remove any possible impurities on the surface. To evaluate surface sensitivity, the cleaned surfaces were incubated overnight with 11-MUA (10mM in ethanol) to create carboxyl groups. Then, the surfaces were assembled with PMMA (thickness: 3.2 mm) using a DSA layer (thickness: 50 μm), and inlet and outlet tubings were attached using epoxy. All PMMA and DSA surfaces were cut using a laser cutter (Versa LASER). A 100 μL of EDC/NHS (100 mM : 100 mM) was passed through the microfluidic channels to create succinimide groups. Through chemical reactions between amine groups of proteins and succinimide groups, protein G and A/G molecules were immobilized on the surface. Same surface chemistry would be potentially applied to immobilize other biomolecules such as antigen and antibody [12, 13].

Simulation: To predict plasmonic response of the DVD surfaces, 3D computation modeling was also performed through finite element method (FEM).

3. RESULTS AND DISCUSSION

The DVD sensor consisted of plasmonic metasurface surface grating as shown in Figure 1a. Multi-layers of DVD metasurface can be considered as a plasmonic optical nanocircuite at optical frequency, where each of the circuit elements is corresponding to characteristic impedance of layered materials [14]. As the sensor consists of plasmonic surface grating (Figure 1a), the resonance wavelength, λ_{res} of the DVD sensor can be approximated by,

$$\lambda_{res} \approx \frac{p_0}{\sqrt{j^2 + k^2}} \sqrt{\frac{\epsilon_s \epsilon_m}{\epsilon_s + \epsilon_m}} \quad (1)$$

where P_0 = grating periodicity, i, j = grating orders, and ϵ_s and ϵ_m are permittivity of the metal and dielectric medium [9]. DVD plasmonic surface simulated using FEM software (Figure 1b). According to the simulation, a 750 nm of periodicity, 450 nm of width, and 30 nm of grating height were considered on our surfaces. The thickness of Ti (10 nm), Ag (30 nm), and Au (15 nm) were smaller enough for the skin effect, enabling to produce semitransparent metasurface. The optical property of the metal-surface was considered using Johnson and Christy model. Experimental results showed a sharp resonance around 750 nm with air medium. Further, an asymmetric Fano resonance property was observed with DI. As shown in the literature, multi-layer metal-dielectric metasurfaces show coupling between SPR and waveguide modes, therefore exhibiting plasmon-induced transparency (PIT) and Fano resonance property [15]. In addition, plasmonic Fano resonance property of the DVD surface can be considered due to mode hybridization between waveguide (WG) guided mode and SPR mode. The computational results also showed plasmonic response similar to the experimental results (Figure 1d-f).

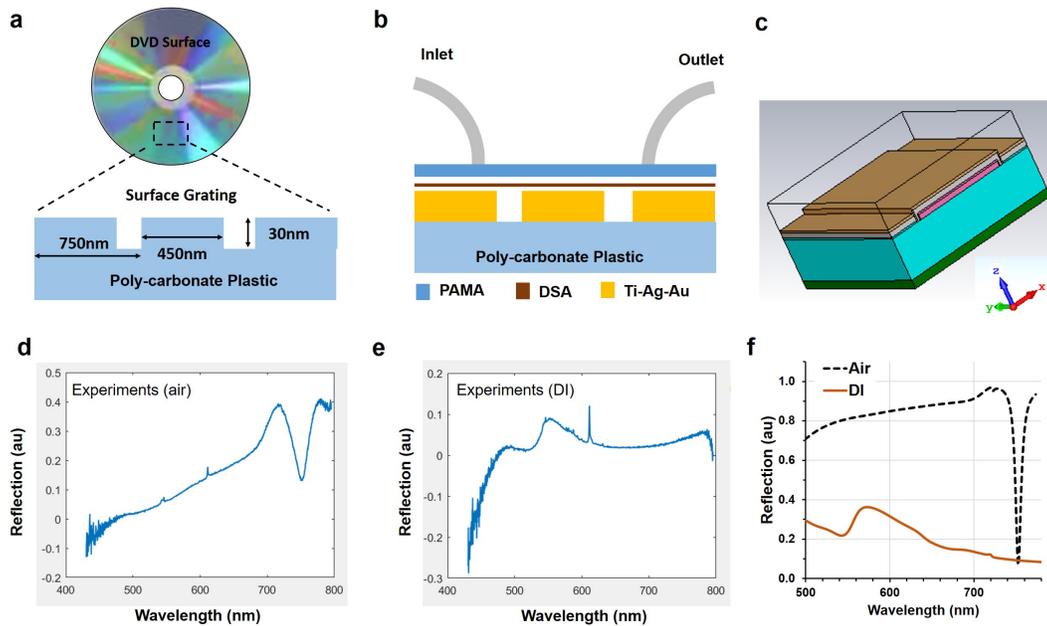


Figure 1: a. DVD surface grating. b. DVD microfluidic plasmonic sensor. c. Computation modeling of DVD sensor. d, e. Experimental plasmonic response of DVD surface with air and DI medium. f. Computational result of DVD surface with air and DI medium.

Optical plasmonic responses of the DVD surface were measured using a customized setup (Figure 2a). The optical setup consisted of a broadband light source (OSL2 Fiber Illuminator, 400-1300 nm), lens, linear polarizer, and optical shutter. Briefly, the light reflects from the DVD surface, passes through the objective (5x), and it is measured on a spectrometer (HR2000+, 342-795 nm). To compare the grating effect on plasmonic response, we also evaluated CD and DVD surfaces as metasurface sensors. By varying glycerol concentrations from 10 to 30%, the plasmonic responses of CD and DVD surfaces were measured, and the DVD surface showed maximum resonance wavelength shifts compared to the CD surface due to the size dependence dielectric function of nanostructure. Therefore, the dielectric value of nanostructure with different sizes surrounded with same dielectric medium will be different [16]. Due to larger features on the CD surface, the dielectric function is lower compared to the DVD surface. Moreover, as glycerol concentration was varied from 10 to 30%, dielectric constant of the medium increased. The maximum peak-wavelength response (λ_{max}) with nanostructure size or aspect ratio (R) and dielectric constant of medium (ϵ_s) can be represented with the following equation [17]:

$$\lambda_{max} = (33.34R - 46.31) \epsilon_s + 472.31 \quad (2)$$

As defined, the real-part of dielectric function of Au nanostructure decreases linearly as a function of wavelength from 500-800 nm and λ_{\max} varies linearly with R.

The sensitivity of DVD plasmonic sensor performance can also be defined by the following equations [18]:

$$\text{Sensitivity, } S = \frac{\Delta\lambda_{res}}{\Delta n_i} = \frac{\lambda \frac{\partial n_{eff}}{\partial n_i}}{n_g} \quad (3)$$

where λ_{res} is the resonance wavelength, n_g is the group refractive index. Due to the evanescent field modified by the dielectric constant of the medium, the sensor sensitivity is strongly dependent on the RI value of surrounding medium property (n_i) and total effective RI value (n_{eff}). As the RI value of dielectric medium increases, n_{eff} will increase, resulting in higher resonance wavelength shifting on DVD plasmonic sensor when higher glycerol concentrations are applied.

Figures 2c,d shows real-time plasmonic response from the DVD and CD surfaces. To observe real-time responses of these surfaces, glycerol solution of various concentrations was applied into the microfluidic channels (flow rate: 5 $\mu\text{l}/\text{min}$) with a syringe pump. After washing the channels with DI, 10% of glycerol was applied and the resonance wavelength shifted from 561.5 to 564.5 nm (Figure 2c, d). Similarly, 20% and 30% glycerol concentrations were applied into the channels and the surface was then washed with DI. In these experiments, we observed 7 nm shift (maximum) at 30% of glycerol concentration. For plasmonic CD device, the wavelength shifts were lower compared to the same concentrations on the plasmonic DVD surface.

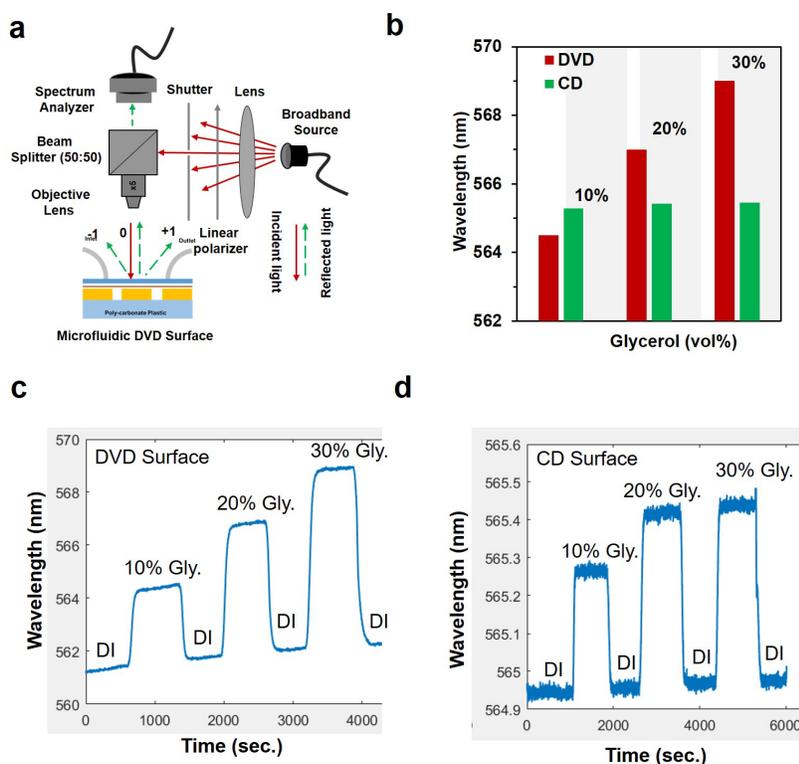


Figure 2: a. Optical setup. b. Plasmonic responses on the DVD and CD surfaces. c, d. Real-time plasmonic responses of DVD and CD surfaces.

In the surface modification experiments, the surface sensitivity of functionalized DVD plasmonic surface was measured on the setup. First, microfluidic channels were washed with PBS, and then, EDC/NHS solution were introduced into the channels. After PBS washing, EDC/NHS caused a 0.6 nm of wavelength shift. Moreover, Protein G and A/G were also passed through the channel and we observed wavelength shifts of 0.667 ± 0.116 nm and 0.353 ± 0.211 nm, respectively. As the procedure is generic, these process steps can also be used to bind other biotargets such as proteins, antibodies, biomarkers, and so on.

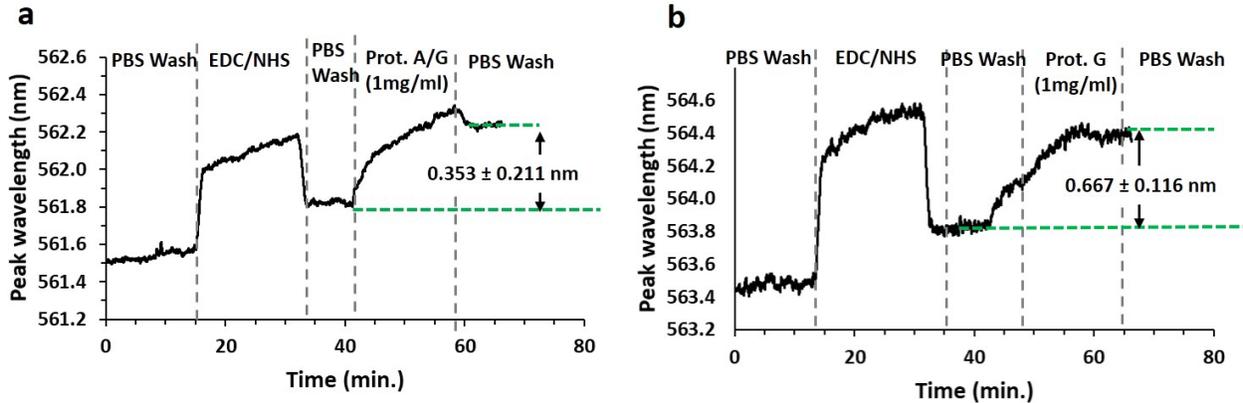


Figure 3: Real-time monitoring of surface sensitivity for monolayer protein A/G (a) and protein G (b) on DVD plasmonic surface.

4. CONCLUSION

In this study, we designed a DVD surface as an inexpensive, label-free plasmonic sensor and characterized the sensor properties through using bulk and surface sensitivities. The DVD surface showed nearly two-fold higher wavelength shifts compared to the CD plasmonic surfaces at 30% glycerol concentration. Therefore, we continued with the DVD surface to monitor real-time binding of biotarget molecules (Protein G and A/G). We performed three replicates for Protein G and protein A/G at 1 mg/mL concentration. Protein G resulted in 0.667 ± 0.116 nm, whereas Protein A/G caused 0.353 ± 0.211 nm of wavelength shift. Statistical assessments indicated that there was no difference between the results of Protein G and Protein A/G at 1 mg/mL concentration ($n=3$, $p>0.05$). Overall, the presented DVD plasmonic sensing platform could be potentially used to detect other biotargets, such as on proteins, antigens, and antibodies, viruses, and cancer biomarkers.

5. ACKNOWLEDGEMENTS

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