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Self-aligned colocalization of 3D plasmonic nanogap arrays for ultra-sensitive surface plasmon resonance detection

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ABSTRACT

We report extremely sensitive plasmonic detection that was performed label-free based on the colocalization of target DNA molecules and electromagnetic hot spots excited at 3D nanogap arrays. The colocalization was self-aligned by oblique evaporation of a dielectric mask over the 3D nanopatterns, which creates nanogaps for spatially selective target binding. The feasibility was experimentally confirmed by measuring hybridization of 24-mer single-stranded DNA oligonucleotides on triangular and circular 3D nanogap arrays. We were able to achieve significantly amplified optical signatures that lead to sensitivity enhancement in terms of detectable binding capacity in reference to conventional thin film-based surface plasmon resonance detection on the order of 1 fg/mm^2.

1. Introduction

Ultra-sensitive detection of molecular interactions has always been desired to elucidate macroscopic phenomena on a molecular scale. Extremely diverse detection approaches have been proposed and implemented to measure molecular scale dynamics in a quantifiable manner and in real time. Most of these approaches require fluorescent labels, the use of which is often accompanied by undesired consequences such as label interference and chemotoxicity. Label-free approaches, on the other hand, tend to suffer from low detection sensitivity. One of the representative label-free detection techniques that have been widely used to characterize molecular interactions is surface plasmon (SP) resonance biosensing, which allows quantitative real-time monitoring and convenient measurement of kinetic coefficients associated with a specific molecular interaction. Despite widespread use of SP resonance (SPR) biosensors, the detectable limit of binding capacity of traditional plasmonic sensing is relatively poor on the order of 1 pg/mm^2 (Campbell and Kim, 2007), which lags far behind recently emerging other label-free detection methods such as those based on whispering gallery modes (Vollmer and Arnold, 2008). For this reason, enhancement of detection sensitivity for advanced plasmonic sensing has its own long history studied with many approaches to date (Nelson et al., 1996; He et al., 2000; Wu et al., 2004; Chien and Chen, 2006; Malic et al., 2007; Yao et al., 2008; Lee et al., 2012a).

The focus of our group in this direction has been to localize evanescent waves on the surface to produce electromagnetic field amplification called hot spots for enhanced plasmonic detection sensitivity (Byun et al., 2005; Kim, 2006; Kim et al., 2006, Yu et al., 2013). While fairly successful compared to traditional thin film-based detection, simple localization of fields to create electromagnetic hot spots by surface modulation has been found much less efficient than conjugation-based amplification that uses metallic nanoparticles (NPs) (Kim et al., 2009; Moon et al., 2010; Krishnan et al., 2011; Law et al., 2011; Moon et al., 2012). Recent studies using 2D linear nanopatterns suggest that spatial distribution of a biomolecular interaction in the localized evanescent fields may be extremely important for effective enhancement of the detection sensitivity (Hoa et al., 2009; Byun et al., 2009; Ma et al., 2010; Kim et al., 2012). Collocation of localized fields and molecular interactions in the same physical space is referred to as colocalization, which is often used to represent the spatial overlap between two different molecules. Spatial colocalization of target biomolecules and evanescent fields was combined with NP-based amplification and showed an increase of plasmonic signals by two orders of magnitude per unit target volume (Oh et al., 2011). Use of NPs, however, has a disadvantage of turning plasmonic detection effectively into a labeled sensing technique.

In this study, we investigate the sensitivity enhancement achievable with colocalized plasmonic detection between target and field localization for hybridization of complementary single-stranded DNA (ssDNA) molecules as a target biointeraction. While evanescent fields may be localized by plasmonic nanostructures, actual implementation of colocalization can be tricky: for example,

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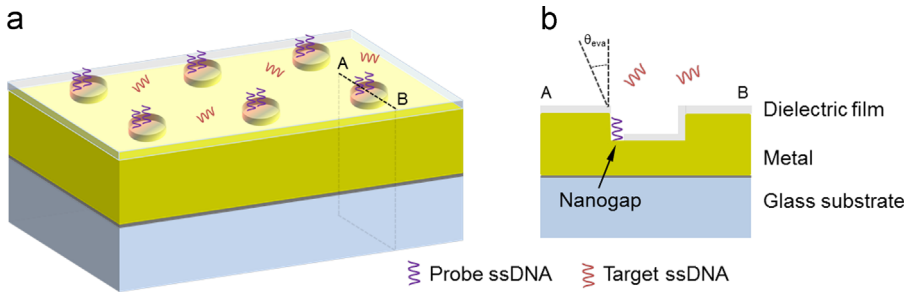

Fig. 1. (a) Schematic illustration of nanogap-based colocalization of DNA hybridization. (b) Device profile across the dashed line (A–B) shown in (a). Schematics not in scale.

techniques such as selective passivation and dip-pen nanolithography may be used. With these techniques, however, perfect registration of target molecules with localized fields would be extremely difficult. Our approach here for implementing colocalized plasmonic detection between target molecules and localized fields is to use oblique evaporation of a thin film mask layer over metallic nanostructures for self-aligned colocalization, as illustrated in Fig. 1. Oblique evaporation was previously used to create nanoscale gaps largely for molecular electronic devices (Kubatkin et al., 2003; Kumagai et al., 2009). Recently, oblique evaporation has been used to produce hot spots for enhanced optical characteristics, for example, to implement improved SERS platforms (Theiss et al., 2010; Siegfried et al., 2011). Here in this work, a nanogap is formed as a result of shadowing at ridges of nanostructures, which provides an access to an underlying metal thin film and substrate, for creating extremely sensitive SPR biosensors. Poor step coverage of evaporation is preferred for this purpose to other deposition techniques, such as sputtering and chemical vapor deposition (Campbell, 2001). Because electromagnetic hot spots are formed at the sharp ridges of metallic nanostructures, if target molecules bind only at nanogaps formed at ridges, targets and electromagnetic fields can be self-aligned for colocalization. As an example of preferential binding, target molecules can bind to metal surface preferentially by thiolation or to dielectric surface by siloxane-based immobilization (Gerdon et al., 2009; Kim et al., 2012). We use 3D nanoapertures for efficient localization of fields and target molecules in an area much smaller than we would produce with 2D patterns. In the non-colocalized detection without nanogaps, DNA hybridization would occur on all the metal surface and detection of target molecules is not as efficient as colocalized detection because majority of target distribution is located where electromagnetic fields are weak. In other words, colocalized SPR detection does not improve the limit of detection of the instrument directly: rather, colocalization selectively amplifies the optical signatures of a target interaction. While there have been many studies to take advantage of localized SPR produced by nanogaps (Kubo and Fujikawa, 2010; Clark and Cooper, 2011; Wu et al. 2012), these studies did not implement colocalization of biomolecular interactions with field localization for SPR biosensing. Note that substrates are usually rotated during evaporation for improved step coverage. Numerical results indicate that the non-uniformity due to oblique evaporation in the dielectric mask layer does not produce a significant effect on the plasmonic near-field distribution (Supplementary Information 1).

2. Methods and materials

2.1. Nanogap fabrication

The overall fabrication procedure is illustrated in Fig. 2. The fabrication starts with evaporation of a 2-nm thick chrome adhesion layer and a 40-nm thick gold film (②) on an SF10 glass substrate (①). 2D grating and 3D nanoarray patterns were defined by electron-beam lithography. For e-beam lithography, we used a negative resist ARN-7520 (Allresist™, Strausberg, Germany) for 3D patterns and poly(methyl methacrylate) (PMMA) e-beam resist (Allresist™) for linear gratings (③). Grating structure was patterned at $\Lambda = 400$ nm. Nanoarrays of equilateral triangles and circles were patterned at $\Lambda = 2\,\mu$m with 600-nm side length (triangles) or 600-nm diameter (circles) (④). The array period was varied for triangular patterns to change the number of nanogaps under detection and thus the number of probe nucleotides that participate in the hybridization. The resist was developed (⑤), followed by the deposition of gold ($d_g = 20$ nm) (⑥) and removal of e-beam resist for lift-off (⑦). For colocalization, an ITO dielectric layer was e-beam evaporated obliquely at an angle θ_{eva} (⑧). The angle was varied at 30°, 45°, and 60° to change the nanogap area. Near-field calculation shows that reduced ITO thickness increases detection sensitivity, yet degrades signal-to-noise ratio (Supplementary Information 2). Based on this result and considering the deposition coverage and precision, ITO layer thickness was selected as 5 nm. In order to enhance the adhesion of ITO, the evaporator chamber was heated for annealing effect at about 200 °C during the oblique evaporation process. ITO is chemically and optically stable to provide good sensor stability, such that degradation in the SPR detection occurs in weeks in atmosphere and days in water ambience (Szunerits et al., 2008). For 2D linear grating-based colocalization, the fabrication procedure is provided in Supplementary Information 3.

2.2. Optical set-up

The optical set-up is illustrated with detailed explanation in Supplementary Information 4.

2.3. DNA preparation and non-specific detection using negative controls

Details of DNA hybridization and non-specific detection using negative controls can be found in Supplementary Information 5.

3. Results and discussion

3.1. Estimation of nanogap areas

For a 2D rectangular grating with thickness d_g, the relative nanogap area G_Λ per unit period is proportionate to

$$G_\Lambda = d_g(1 + \tan\theta_{eva}), \quad (1)$$

where θ_{eva} denotes the evaporation angle shown in Fig. 1b. In other words, the amount of molecules in an interaction can be varied by changing the evaporation angle, thus the nanogap size. The amount can also be varied by changing the number of patterns per unit surface area based on the assumption that the number is