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Enhanced SPR response from patterned immobilization of surface bioreceptors on nano-gratings

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ABSTRACT

In this report, nano-gratings with guided adsorption of biomolecules are investigated as new transducer elements or biointerfaces for surface plasmon resonance biosensor technologies. SPR biosensors are of particular interest due to the interaction between the electromagnetic fields and periodic nano-structures. In this article, sensitivity enhancement is demonstrated for a surface plasmon resonance interface, in a Kretschmann's configuration, featuring nano-gratings combined with nano-patterned immobilization of surface bioreceptors. The fabrication of this enhanced biointerface is demonstrated using a combination of metal lift-off and self-assembled monolayers. Rigorous coupled-wave analyses point to an increase in SPR angular response for the immobilization of surface bioreceptors onto areas of the nano-corrugated surface exhibiting high electromagnetic field intensity. Experimental measurements of the immobilization of anti-TNF-α antibody as a model bioreceptor using an imaging-SPR technique show a 3 times increase in angular resonance response from nano-grating surfaces with functionalized mesas compared to a planar surface or to a uniformly functionalized nano-grating surface. Furthermore, results also show an increased detection of TNF-α due to the increased accessibility to the adsorbed bioreceptors on the nano-gratings.

1. Introduction

Today, the development of surface plasmon resonance (SPR) immunosensors or biosensors is increasingly focused on the integration of the system components for in-field biomedical, food and environmental applications. The interest in the SPR technology lies in its label-free and real-time biomolecular analysis. In many cases, the detection of low-molecular weight biomolecules (proteins, DNA) at low concentrations is sought, raising significant challenges for the design of various integrated biosensor components including the optical system, data-analysis method, and in particular the functionalized biointerface. For the latter, the literature presents several examples of surface engineering techniques developed to increase the surface binding affinity, capacity and specificity of SPR biosensors and other immunosensors. Different strategies are described ranging from three-dimensional matrices of polymers with multiple bioreceptor attachment sites to colloidal-enhanced surfaces (Hu et al., 2004; Lofas, 1995; Matsui et al., 2005). Periodic nano-structured surfaces are also recently introduced as novel SPR transduction substrates due to their unique optical properties.

Periodic structures (gratings) have been used in SPR biosensing since its early development. In some SPR systems, metallic gratings are employed to couple incident light into the surface plasmon waves. The gratings provide a mechanism to increase the illumination light momentum (Knoll, 1998). Today, interest in nano-structures also stems from the phenomenon of localized surface plasmon resonance of metallic particles on surfaces. These substrates are known to exhibit absorbance spectra that are sensitive to refractive index near the particles surface, and to the particle size and distribution (Haes and Van Duyne, 2004; Stuart et al., 2005). The latter can be used for biosensing applications once the particles are functionalized to capture a given biomolecule of interest.

Surface plasmon resonance biosensors utilise the excitation of a propagating electromagnetic (EM) wave (surface polariton or plasmon wave) to sense a binding event, the adsorption of the biomolecules of interest, at a metal-dielectric interface. Simply explained, at a metal-dielectric interface, the free electrons of the metal surface can support a collective oscillation, generating an associated EM wave (Raether, 1988). The oscillations are excited by an external illumination provided that the photon momen-

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tum is matched to the wave vector of the surface plasmon wave. This is referred to as the resonance condition or resonance point. The latter is strongly dependent, among other system parameters, on the refractive index of the dielectric medium. Free-space photons require additional momentum in order to meet this resonance condition. The required momentum can be provided by a high index prism or as mentioned a grating structure. In the conventional prism coupler system (Kretschmann's configuration), the matching occurs at a given illumination wavelength and incident angle. As the refractive index of the dielectric medium changes, due to the adsorption of biomolecules, the resonance condition also changes, resulting in a measured resonance wavelength or angle shift. By tracking the resonance shift, one can correlate real-time measurements to the adsorption of the biomolecules. The plasmon waves are surface bound phenomena; as such, only local refractive index changes of the medium, close to the metal-dielectric interface are sensed. For a biosensor, the metal-dielectric interface is functionalized with surface bioreceptors, chosen for their specific affinity toward a given biomolecule to provide the biorecognition feature.

Functionalization techniques for SPR interfaces are numerous. These include long polymer chains of dextran (polysaccharide) featuring multiple bioreceptor attachment sites (Lofas, 1995), porous matrices with increased surface areas (Oh et al., 2006), and uniform self-assembled monolayers (Ulman, 1996). The patterning of the surface chemistry at the microscale and nanoscale is also extensively explored in the literature. Micro-contact printing, dip-pen nanotechnology, laser ablation and many others techniques are described (Coyer et al., 2007; Kirkwood et al., 2007; Lee et al., 2002; Rundqvist et al., 2007). Generally, they are employed to create substrates for cell-based biosensors and arrayed assays (DNA-chip, Bio-chip).

This article focuses on a novel functionalized biointerface featuring nano-structures, specifically zero-order gratings (nano-grating), placed on the medium or sample side of the metallic surface of a Kretschmann's SPR prism coupler configuration. Note that in this application, the nano-gratings are not used to couple light into the surface plasmon waves. Nano-gratings can perturb the propagation of the evanescent surface plasmon wave and generate a bandgap in its dispersion relation (Benahmed and Ho, 2007; Bonod et al., 2008; Fischer et al., 1994; Pincemin and Greffet, 1996). The bandgap represents a range of wavelengths or incident angles for which an incident light cannot excite a surface plasmon wave at the metal-dielectric interface (Barnes et al., 2003). It has been shown theoretically that for a biosensor operating near the bandgap, a multiple-fold increase in sensitivity is obtained when compared to a planar interface (Alleyne et al., 2007). Furthermore, the evanescent plasmon fields are redistributed on the surface such that areas of concentrated field intensity are created. This paper explores the possibility of further extending the sensitivity of this approach by guiding the immobilization of surface bioreceptors to specific sites on the nano-grating surface. The purpose is to create a patterned periodic immobilization to enhance the metallic nano-grating effect by concentrating the adsorption of bioreceptors on to areas of increased field intensity. In the following sections, the fabrication of the functionalized surface is first described. The technique uses a combination of electron-beam lithography, metal lift-off and self-assembled monolayers. Numerical studies are also carried out to predict the surface plasmon response and the field distribution. Finally, measurements on an imaging-SPR are carried out, using anti-TNF-α/TNF-α as a model bioreceptor/antigen, on the different fabricated substrates. TNF-α is a cytokine involved in the systemic inflammation response; it has been suggested that TNF-α can be used as a potential biomarker for the diagnosis of various diseases, including sepsis (Carrigan et al., 2004).

2. Materials and methods

2.1. Nano-structured SPR surface fabrication

In this paper, the design of a nano-structured and nano-patterned SPR interface employs Kretschmann's configuration given its simplicity and high sensitivity (Homola et al., 1999). The enhanced interface consists of a high index substrate coated with a thin layer gold, on which a binary 250 nm period gold nano-grating is introduced. The nano-gratings are 15 nm deep with 50% duty factor. The duty factor refers to the ratio of the grating mesa's width to its period. In this case, the mesas and troughs are of equal width. Once fabricated, the nano-structured and functionalized substrates can be placed atop the coupling prism of an existing SPR system for measurements. Surface bioreceptors constituting of antibodies are selectively immobilized exclusively on nano-grating mesas, on the trough or uniformly across the nano-grating and on a reference planar surface for comparative studies (Fig. 1).

The fabrication of the nano-structured and nano-patterned functionalized substrates is realised by electron beam lithography and a metal lift-off process. The details of the fabrication process have been presented in an earlier work (Hoa et al., 2008), which focuses on the characterization of nano-patterned surfaces using scanning near-field optical microscopy. The fabrication technique allows for the simultaneous fabrication of the gold nano-gratings and the selective immobilization of surface bioreceptors on the nano-grating mesa or trough with the passivation of substrate and the unfunctionalized nano-grating features. Four variations of the patterned interfaces are produced in this study, as shown in Fig. 1.

The SPR interfaces are fabricated on a high index planar substrate of SF11 glass (Schott). The fabrication starts with the deposition of a gold layer on the substrate with 3 nm of Cr for adhesion, followed by the deposition of a poly(methyl methacrylate) (PMMA) 150 nm bi-layer resist. The resist is then patterned by an electron-beam (LEO VP electronic microscope at 30 pA and 20 keV) over a 250 μm × 250 μm area with equally spaced 125 nm wide lines. For the reference planar interface (Fig. 1(d)), the entire 250 μm × 250 μm is exposed. The PMMA is then developed in isopropanol to reveal the written patterns. A 1 min quick oxygen plasma is applied to clean the surface. 3 nm of Cr is deposited; follow by a second deposition of gold, the thickness of which determines the nano-grating height.

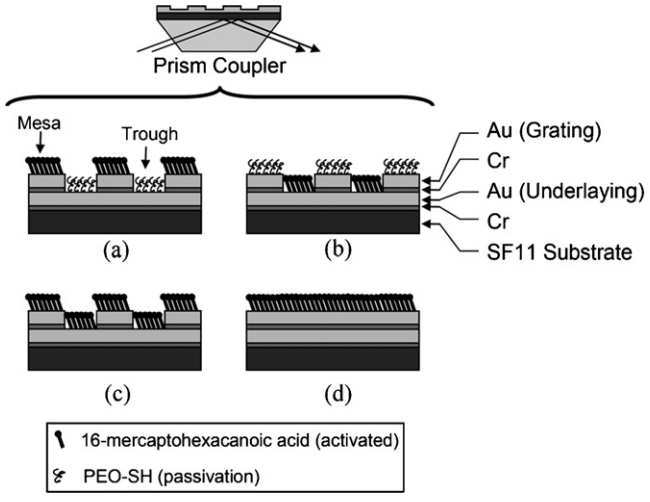

Fig. 1. Four biointerface designs (a) binary nano-grating with surface bioreceptors on the nano-grating mesas (b) with surface bioreceptors in the nano-grating troughs (c) uniformly distributed (d) reference surface with planar interface and functionalization.