Periodic nanohole arrays with shape-enhanced plasmon resonance as real-time biosensors

Antoine Lesuffleur, Hyungsoon Im, Nathan C. Lindquist, and Sang-Hyun Oh[a]

Laboratory of Nanostructures and Biosensing, Department of Electrical and Computer Engineering, University of Minnesota, Twin Cities, Minneapolis, Minnesota 55455

The authors report a combination of the enhanced transmission effect and shape resonances in a periodic array of nanoscale double-hole structures in a gold film to enhance the detection sensitivity of surface plasmon biosensors. Finite-difference time-domain calculations are used to quantify field enhancement at the apexes of the double-hole structure. The double-hole array was used to measure the formation of a self-assembled monolayer and for real-time sensing of protein adsorption onto a gold surface. This result demonstrates the potential to integrate propagating surface plasmons and localized shape resonances to improve real-time biosensors. © *2007 American Institute of Physics.*
[DOI: 10.1063/1.2747668]

Surface plasmon resonance (SPR) provides an attractive path toward quantitative biomolecular analysis and drug discovery processes, wherein the local increase in the refractive index due to molecular binding near a metal-dielectric interface can be optically monitored in a real-time, label-free mode.[1,2] In conventional SPR instruments, e.g., BIAcore™, a convergent light cone illuminates the detection spot via prism coupling in total internal reflection mode, known as the Kretschmann configuration,[3] and the angular distribution of the reflected light intensity is measured in real time. This setup, however, is not easily amenable to miniaturization and hinders the use of high numerical aperture imaging optics that is required for multiplex SPR sensing.

Recent discovery of the surface plasmon-mediated extraordinary optical transmission effect through periodic nanohole arrays[4] shows potential to miniaturize a label-free, real-time biosensing element and realize high-throughput, multiplex SPR sensing on a microarray.[5,6] Since this effect is sensitive to changes in the refractive index at the metal-dielectric boundary (viz., within ∼200 nm of the surface), this signal transduction mechanism can be exploited to detect small molecule binding events in real time. In contrast with the bulky prism coupling used in the Kretschmann setup, the nanohole array can provide (1) a collinear, transmission measurement setup that greatly simplifies and miniaturizes optical design, alignment, and imaging; and (2) a small-footprint sensing probe ($\approx 10 \times 10 \ \mu m^2$), which enables a high packing density for multiplex array sensing. The spectral selectivity of the enhanced transmission matches with Bragg resonances of surface plasmon (SP) on a periodically modulated lattice on the metallic surface. For normal incidence onto a square-symmetry periodic lattice, the transmission maxima occur at the wavelengths satisfying a two-dimensional grating coupling condition for SP,[7]

$$\lambda_{SP}(i,j) = \sqrt{\frac{\varepsilon_d \varepsilon_m}{\varepsilon_d + \varepsilon_m}} \frac{P}{\sqrt{i^2+j^2}}, \qquad (1)$$

where P is the periodicity of the array, the integers (i,j)

represent the Bragg resonance orders, and ε_m and ε_d are the dielectric functions of the metal and dielectric, respectively.

Static measurements of changes in the refractive index due to molecular adsorption on a periodic array of circular nanoholes were performed by Brolo *et al.*[5] However, real-time sensing of molecular adsorption, which is the ultimate goal of the SPR sensor, was not demonstrated in that work. More recently, it was shown that the shape of the nanoholes in metals can modulate their optical transmission properties.[8–11] Lesuffleur *et al.* demonstrated that the presence of sharp apexes in nanoscale double-hole structure can boost the local field intensity, transmission efficiency, and polarization selectivity compared to circular nanohole arrays.[10] It has been demonstrated that such an effect involves localized SPR (LSPR).[12] Haes and Van Duyne established the use of LSPR spectroscopy of Ag nanoparticles for sensing application,[13] and work by McFarland and Van Duyne[14] showed that the LSPR in a single silver nanoparticle, which has shorter decay length than propagating SPR on a planar surface, can significantly enhance the detection sensitivity of small molecules.

In this letter, we present the demonstration of combining the enhanced transmission effect and the shape resonances of a nanohole array to show the real-time, label-free detection sensitivity for the adsorption of biomolecules.

Figure 1 shows a scanning electron microscope image of a nanoscale double-hole array, which was milled using a FEI dual-beam focused ion beam through a 100-nm-thick gold film with a 5 nm Cr adhesion layer on a glass substrate. The double-hole structure consists of two overlapping circular holes (200 nm diameter, 190 nm center spacing) to produce two apexes in between. Each pattern consists of a 40×40 nanohole array with a periodicity of 600–800 nm. A tungsten-halogen lamp illuminated the nanohole array through a linear polarizer and a $50\times$ microscope objective lens, and the transmitted light was collected using a fiber-optic spectrometer. Figure 1 (inset) shows the normalized transmission spectrum from a nanohole array (600 nm periodicity) when the polarization of the incident light is along the (0,1) Bragg resonance direction. Two peaks were observed at 540 and 645 nm, corresponding to $\lambda_{SP}(1,1)$ and $\lambda_{SP}(0,1)$ at the air-gold interface, respectively. As expected,
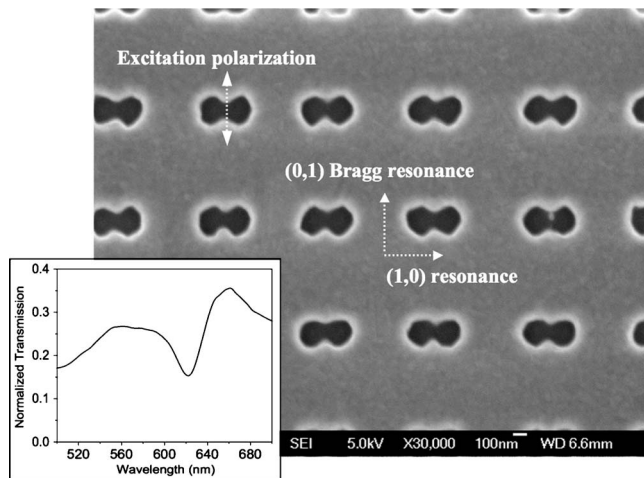
a)Electronic mail: sang@umn.edu

FIG. 1. Scanning electron microscopy picture of a double-hole array with a 800 nm periodicity. The diameter of the circular hole is 200 nm and the center-to-center hole distance is 190 nm. (Inset) Normalized transmission spectrum of a double-hole array with a 600 nm periodicity and the light polarization along the (0,1) Bragg resonance direction.

$\lambda_{SP}(0,1)$, which is aligned to the polarization of the excitation, shows stronger transmission enhancement and a sharper resonance peak than the oblique (1,1) Bragg resonance.

The refractive index sensitivity of the double-hole array was measured with air, water ($n=1.33$), and acetone ($n=1.36$) using a 150 μm height flow cell attached to the gold surface. The experimental results in Fig. 2(a) illustrate that the $\lambda_{SP}(0,1)$ peak was shifted by 200 nm in water, which corresponds to a bulk refractive index sensitivity of 600 nm/RIU (RIU denotes refractive index unit), whereas $\lambda_{SP}(1,1)$ shows a smaller shift of 270 nm/RIU. The bulk refractive index sensitivity of the double-hole array is larger than the previously reported circular nanohole arrays (400 nm/RIU) (Ref. 5) and silver nanoparticle sensor (235 nm/RIU).[14]

After recording the transmission spectrum, the nanohole array was incubated for 3 h in a 4 mM self-assembled monolayer (SAM) 11-amino-1-undecanethiol hydrochloride, rinsed in water and dried. Figure 2(b) shows the spectral measurements performed in the air. With the introduction of SAM, [1.6 nm in thickness with the refractive index of 1.45 (Ref. 15)], shifts of $\Delta\lambda_{SP}(0,1)=6\pm1$ nm and $\Delta\lambda_{SP}(1,1)$ $=3\pm1$ nm were measured. In comparison, circular nanohole arrays[5] showed $\Delta\lambda_{SP}(0,1)=4\pm1$ nm for Au modified with a monolayer of 11-mercaptoundecanoic acid (thickness $=1.7$ nm and $n=1.45$). The expected theoretical shift can be calculated using the following equation:[16]

$$\Delta\lambda_{max} = m\Delta n[1 - \exp(-2d/l_d)], \qquad (2)$$

where m is the bulk refractive index response, 600 nm/RIU for $\Delta\lambda_{SP}(0,1)$ and 270 nm/RIU for $\Delta\lambda_{SP}(1,1)$, Δn is the change in refractive index induced by the adsorbate, d is the effective adsorbate layer thickness, and l_d is the characteristic decay length of the evanescent electromagnetic (EM) field perpendicular to the interface. At 650 nm wavelength, the calculated decay length of a propagating SP on a smooth air/gold interface is $l_d=210$ nm, which predicts $\Delta\lambda_{SP}(0,1)$ $=3.8$ nm and $\Delta\lambda_{SP}(1,1)=2$ nm. However, these simple calculations do not take account of LSPR, which can significantly shorten the decay length near the apexes. Finite-
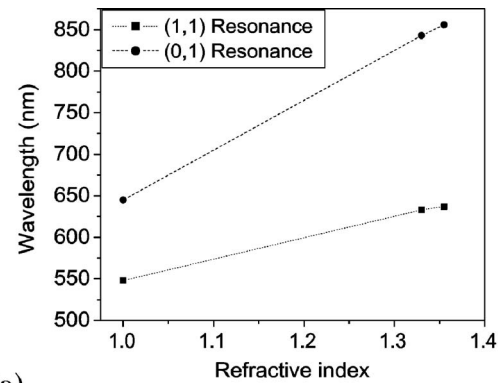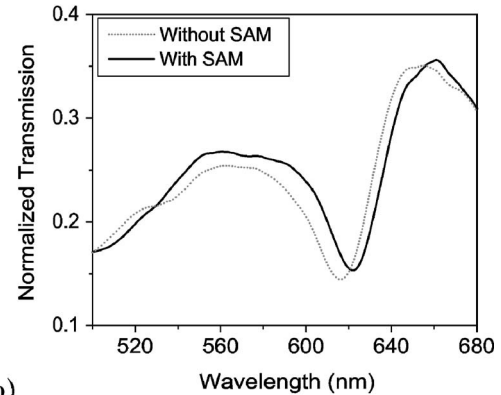
FIG. 2. (a) Wavelength of resonance vs refractive index of the dielectric for the (1,1) and (0,1) Bragg resonances. (b) Spectra of the double-hole array with a 600 nm periodicity in the air and after binding of a self-assembled monolayer of molecules on the surface.

difference time-domain (FDTD) simulations were performed to calculate the characteristic decay length near double-hole structures and estimate the detection sensitivity. The grid spacing was 5 nm in all three dimensions and periodic boundary conditions were used. Figure 3 shows FDTD calculation of the EM-field distribution for the (0,1) Bragg resonance. The decay length in the vicinity of the apexes is reduced to $l_d=20$ nm. Using this FDTD-simulated decay length at the apexes, $\Delta\lambda_{SP}=37$ nm is predicted for molecular adsorption near an ideally sharp apex. The field enhancement, however, is confined over a small region near the apexes, so it is expected that the short decay length and enhanced adsorbate detection sensitivity at the apexes will not translate into the commensurate enhancement in the overall sensitivity across the entire sensing surface. The measured shift of $\Delta\lambda_{SP}(0,1)=6$ nm indicates that the double-hole array combines attributes of both propagating SPR and LSPR. Also, the discrepancy is partly due to rounded apexes.

Although the Kretschmann setup provides higher sensitivity to changes in the bulk refractive index (>3000 nm/RIU) than the double-hole array sensor (600 nm/RIU), the response of the two techniques becomes comparable when measuring short-range changes in the refractive index. This is a result of the much smaller sensing volume offered by LSPR, as the EM-field-decay length is shorter at the apexes. Also, the nanohole array is more easily scaled to a much smaller footprint, which would allow a smaller analyte volume. Furthermore, the double-hole sensor demonstrates $\sim50\%$ improvement in the detection sensitivity compared to the circular nanohole array reported by Brolo *et al.*[5] It demonstrates that LSPR plays an important role in

FIG. 4. Real-time sensing of 0.2% bovine serum albumin (BSA) on the gold surface. Wavelength of the (0,1) resonance vs time for an array with a 600 nm periodicity.
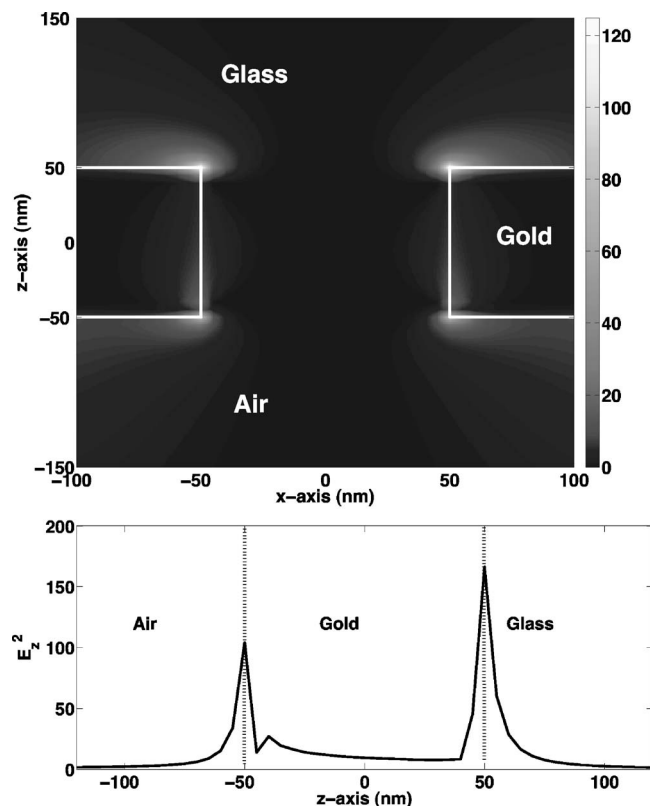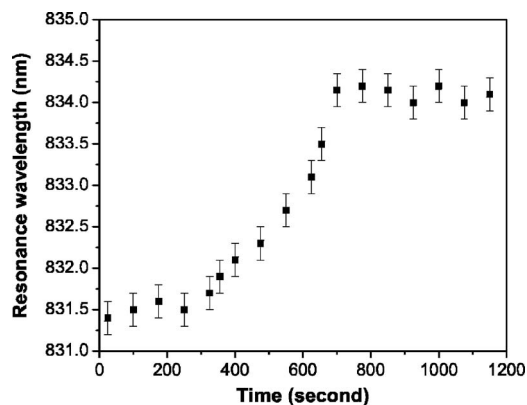
FIG. 3. Finite-difference time-domain (FDTD) calculations of double-hole array with a 600 nm periodicity, 200 nm hole diameter, and 190 nm separation between overlapping holes. (a) *x-z* map of the *z*-component of the electric field. (b) Slice for $x=50$ nm.

improving the sensitivity for thin adsorbate on the surface. The adsorbate detection sensitivity and probing range of double-hole array can be further improved by optimizing the hole shape, sharpness of apexes, and the periodicity of the array. Such work is in progress.

Figure 4 shows the real-time $\lambda_{SP}(0,1)$ response of a nanohole sensor exposed to 0.2% bovine serum albumin (BSA) in phosphate-buffered saline solution in a flow cell (150 μm in height). A transmission spectrum was recorded every 15 s over the course of 1 h. The response of $\Delta\lambda_{SP}(0,1)=3$ nm was observed after 1000 s, when the response nearly saturates. This experiment demonstrates the potential of the nanohole array in a transmission optical setup to perform real-time, label-free sensing of protein interactions.

The shape-enhanced LSPR in the periodic double-hole array improves its refractive index sensitivity, transmission efficiency,[17] sharpness of resonance, and polarization selectivity compared to circular nanohole array. These are all important parameters to enhance the adsorbate detection sensitivity, resolution, and signal-to-noise ratio of real-time multiplex biosensors. Furthermore, the sharp apexes in the double-hole structures provide a path to seamlessly integrate real-time SPR biosensing and surface-enhanced Raman scattering (SERS) spectroscopy[18,19] on the same chip.

In summary, we have demonstrated that the double-hole structure in a gold film improves the bulk refractive index sensitivity (600 nm/RIU) and adsorbate detection sensitivity compared to circular nanohole arrays. The enhanced sensi-tivity of the double-hole structures is attributed to the existence of LSPR and the short decay length of evanescent field at the apexes, which was confirmed by FDTD simulations. The nanohole sensor has been used for real-time sensing of BSA adsorption onto a gold surface. The utilization of nanohole shape resonances for biosensing is promising, since they add new parameters to optimize the detection sensitivity and open up possibilities to integrate other detection modalities, especially SERS spectroscopy, on the same platform.

[1]B. Liedberg, C. Nylander, and I. Lundstrom, Sens. Actuators **4**, 299 (1983).
[2]M. A. Cooper, Nat. Rev. Drug Discovery **1**, 515 (2002).
[3]H. Raether, *Surface Plasmons* (Springer, Berlin, 1988), p. 11.
[4]T. W. Ebbesen, H. J. Lezec, H. F. Ghaemi, T. Thio, and P. A. Wolff, Nature (London) **391**, 667 (1998).
[5]A. G. Brolo, R. Gordon, B. Leathem, and K. L. Kavanagh, Langmuir **20**, 4813 (2004).
[6]Y. Liu, J. Bishop, L. Williams, S. Blair, and J. Herron, Nanotechnology **15**, 1368 (2004).
[7]H. F. Ghaemi, T. Thio, D. E. Grupp, T. W. Ebbesen, and H. J. Lezec, Phys. Rev. B **58**, 6779 (1998).
[8]R. Gordon, A. G. Brolo, A. McKinnon, A. Rajora, B. Leathem, and K. L. Kavanagh, Phys. Rev. Lett. **92**, 037401 (2004).
[9]K. J. Klein Koerkamp, S. Enoch, F. B. Segerink, N. F. van Hulst, and L. Kuipers, Phys. Rev. Lett. **92**, 183901 (2004).
[10]A. Lesuffleur, L. K. S. Kumar, and R. Gordon, Appl. Phys. Lett. **88**, 261104 (2006).
[11]A. Lesuffleur, L. K. S. Kumar, and R. Gordon, Phys. Rev. B **75**, 045423 (2007).
[12]E. X. Jin and X. Xu, Appl. Phys. B: Lasers Opt. **84**, 3 (2006).
[13]A. J. Haes and R. P. Van Duyne, J. Am. Chem. Soc. **124**, 10596 (2002).
[14]A. D. McFarland and R. P. Van Duyne, Nano Lett. **3**, 1057 (2003).
[15]Q. Wang and P. W. Bohn, Thin Solid Films **513**, 338 (2006).
[16]L. S. Jung, C. T. Campbell, T. M. Chinowsky, M. N. Mar, and S. S. Yee, Langmuir **14**, 5636 (1998).
[17]L. K. S. Kumar, A. Lesuffleur, M. C. Hughes, and R. Gordon, Appl. Phys. B: Photophys. Laser Chem. **84**, 25 (2006).
[18]K. A. Willets and R. P. Van Duyne, Annu. Rev. Phys. Chem. **2007**, 267.
[19]A. Lesuffleur, L. K. S. Kumar, A. G. Brolo, K. L. Kavanagh, and R. Gordon, J. Phys. Chem. C **111**, 2347 (2007).