J|A|C|S COMMUNICATIONS

Published on Web 12/30/2008

Attomolar Protein Detection Using in-Hole Surface Plasmon Resonance

Jacqueline Ferreira,[†,‡] Marcos J. L. Santos,[†,‡] Mohammad M. Rahman,[†] Alexandre G. Brolo,*[,†] Reuven Gordon,[§] David Sinton,[||] and Emerson M. Girotto[‡]

Department of Chemistry, University of Victoria, P.O. Box 3065, V8W 3V6, Victoria, BC, Canada, Universidade Estadual de Maringa, Departamento de Quimica, Av. Colombo 5790, 87020900 Maringa, PR, Brazil, Department of Electrical and Computer Engineering, University of Victoria, BC, V8W 3Z8, Victoria, BC, Canada, and Department of Mechanical Engineering, University of Victoria, BC, V8W 3P6, Victoria, BC, Canada

Received October 3, 2008; E-mail: agbrolo@uvic.ca

Surface plasmon resonance (SPR) is the most established of the label-free methods to monitor surface-binding, presenting many advantages in terms of sensitivity, speed, and reliability.[1,2] The most common commercial SPR technology operates in reflection (Kretschmann configuration), which limits its potential to miniaturization, multiplexing, and integration in microfluidic systems. A new SPR approach based on the extraordinary optical transmission (EOT) through nanohole arrays in metal films[3] has been proposed to overcome those limitations.[4−6] The normal transmission geometry of EOT is simpler than the Kretschmann arrangement. The transmission setup is also more suitable for miniaturization and implementation of multiplex detection by imaging SPR.[7,8] However, the EOT approach provides a smaller sensor output sensitivity than the Kretschmann configuration. Several groups have addressed this problem by either optimizing the hole-shape or by implementing more efficient detection schemes.[9−11] In all these cases, the focus was in optimizing the response of the device to refractive index changes.

Here we propose an alternative approach where the efficiency of the sensor is enhanced by confining minute amounts of the analyte inside the nanoholes. The idea is to use only the inside walls of the nanoholes as sensing areas (Figure 1), while blocking the surface outside the holes. A planar gold slide was coated with a 50 nm layer of SiO_x, using a sol−gel procedure previously described to encapsulate nanoparticles.[10] Subwavelength hole-arrays were milled by focused ion beam (FIB) all the way through the SiO_x layer and the gold film. The efficiency of the SiO_x layer in blocking the gold surface was assessed by cyclic voltammetry, which indicated a minimal presence of pinholes.[12] Since the only gold-exposed area was inside the nanoholes, the amount of analyte sensed was reduced by 5 (for the parameters showed in Figure 1) compared to substrate not coated with SiO_x. This is a conservative estimative, because it does not take into account the fact that the SP senses beyond the boundaries of the arrays for the uncoated substrate.[13,14]

Figure 2 displays the transmission spectra of white light through the arrays of subwavelength holes immersed on glucose solutions with different refractive indexes. A red-shift of the maximum transmission is observed as the refractive index of the near-surface layer increases. Considering the thickness of the SiO_x film on the top surface and the evanescent decay of a surface plasmon polariton, we calculated that the sensitivity of the gold surface coated with the oxide to changes in the refractive index of the aqueous environment was reduced to 1% of its value without the oxide layer.

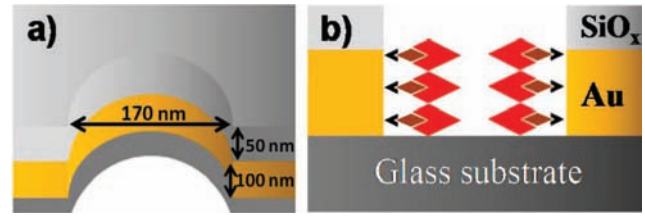

Figure 1. (a) Cross section representation of a nanohole, illustrating the 100 nm gold layer (yellow) sandwiched between the glass substrate and the SiO_x nanolayer (50 nm-thick). (b) Another cross section of a nanohole showing that the molecular binding only occurs inside the holes. The experiments were realized using 15 μm × 15 μm arrays containing 170 nm-diameter nanoholes and a periodicity of 500 nm.

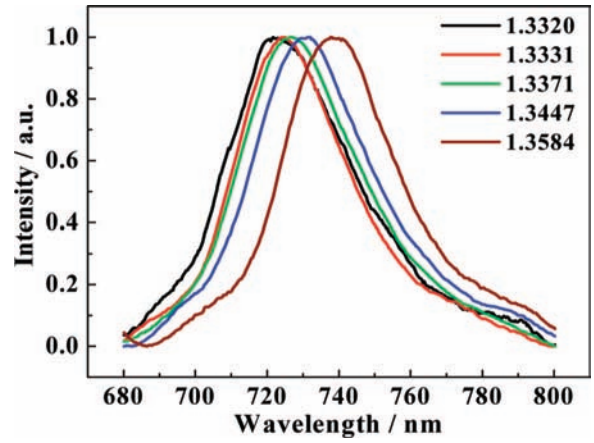

Figure 2. Normalized transmission of white light through an array of nanhoholes immersed in glucose solutions of different refractive indexes.

However, a sensor output sensitivity of 650 nm/RIU (refractive index units) was obtained from Figure 2. Surprisingly, this sensitivity is comparable to the experiments without the SiO_x layer (400 nm/RIU). Therefore, the coating of the top surface did not decrease the sensor output relative to an uncoated surface. This counter-intuitive effect will be later discussed by considering the influence of the refractive index of the adsorbed layer on the properties of the lowest order waveguide mode inside the hole.

In-hole bioaffinity tests were performed using the biotin-streptavidin scheme.[15] The white light transmission through the arrays of nanoholes were measured after each binding step in the presence of flowing PBS through the flow cell. Figure 3 shows the white light transmission spectra obtained after each step. The wavelength shifts ($\Delta\lambda$), calculated relative to the gold surface covered with a monolayer of cysteamine, are plotted in the inset of Figure 3. The SPR from the final modified surface (cysteamine−biotin

[†] Department of Chemistry, University of Victoria.
[‡] Universidade Estadual de Maringa, Departamento de Quimica.
[§] Department of Electrical and Computer Engineering, University of Victoria.
[||] Department of Mechanical Engineering, University of Victoria.

10.1021/ja807704v CCC: $40.75

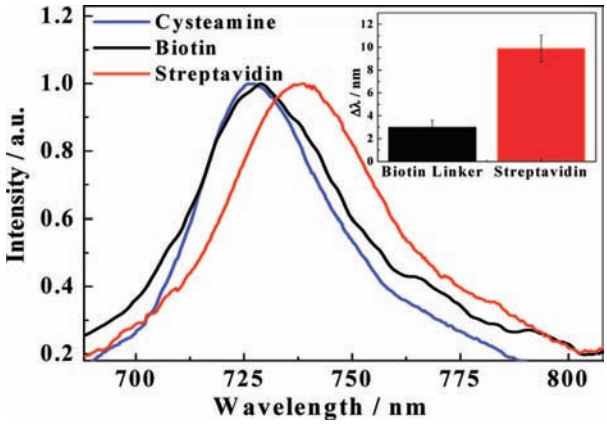

Figure 3. Transmission spectra obtained from an array of nanoholes coated with SiO_x when the gold surface inside the holes was sequentially modified by a monolayer of cysteamine, biotin, and streptavidin. Measured wavelength shifts of the SPR resonance following addition of biotin and streptavidin relative to that of the surface coated only with cysteamine are shown in the inset.

linker−streptavidin) was red-shifted by 10 nm relative to the gold surface covered with only cysteamine. Nonspecific binding of streptavidin on SiO*x* layer was ruled out by negative control experiments. The transmission spectra from unmodified arrays of nanoholes immersed in streptavidin solutions (in PBS) presented no shift relative to the buffer alone.

The inset in Figure 3 summarizes the results from four separate measurements using 16 arrays. The error bars correspond to the standard deviation from these measurements. The array-to-array reproducibility, calculated as the standard deviation of the streptavidin shift from the 16 arrays investigated, was about 20%. Considering a full coverage of 25 nm^2 streptavidin molecules, it is possible to estimate that only 2000 molecules were probed from each hole. Since each array contained 900 holes (30 × 30 nanoholes), the 10 nm shift from Figure 3 was provoked by around 3 attomoles of the protein. This low detection limit was possible because the SiO_x coating did not decrease the sensor output sensitivity of the arrays investigated. The amount of detected protein can be further decreased by using smaller arrays, with no detrimental effect to the sensor output sensitivity.[10,17]

The fact that the SiO_x layer does not strongly affect the sensor output sensitivity can be explained by analyzing the optical mode in the hole. We compared a uniform refractive index change inside the hole with the surface-layer refractive index from the cysteamine−biotin−streptavidin system. By solving the Helmholtz equation for the longitudinal field of the lowest order mode, it was found that a uniform refractive index of 1.3518 (without any surface layer) was equivalent to a 7 nm thick surface layer of cysteamine−biotin−streptavidin (refractive index 1.45) and buffer (refractive index 1.3376) inside the hole. This agrees well with our experiments. The lowest order mode is the only mode considered in these calculations since it dominates the optical transmission process for optically thick films.[16] It is clear that the mode inside the hole is highly sensitive to surface absorption, which is the result of the large longitudinal field component at the surface, as shown in Figure 4. Within the resolution limits of this figure, the same field distribution was observed for a surface layer of cysteamine−biotin−streptavidin and an uniform refractive index of 1.352 (which would yield a 10 nm shift from water, according to the sensor sensitivity obtained from Figure 2).

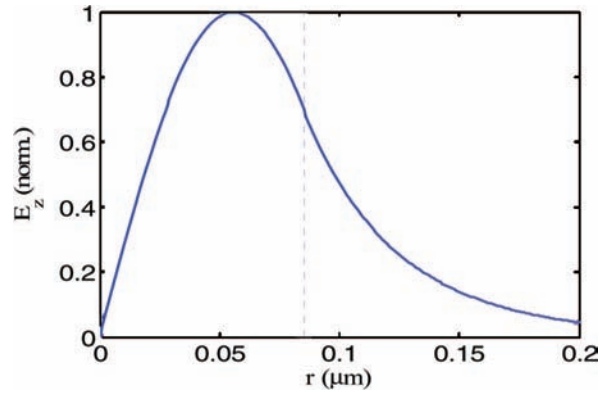

Figure 4. Longitudinal-field radial distribution for lowest-order mode in-hole at $\lambda = 730$ nm. Dashed line shows radius of hole (85 nm). The 7 nm thick cystemaine−biotin−streptivin surface layer ($n = 1.45$) with buffer ($n = 1.3376$) is indistinguishable from a uniform refractive index of 1.352 to within the resolution of this figure. The relative permittivity of Au was −18.

In summary, we have demonstrated that the detection of binding events inside nanoholes allows for good SPR sensitivity and lower limit of detection. The sensitivity of the integrated device output was 650 nm/RIU, which is comparable to previously reported values; however, the in-hole sensing concept allows detection of a smaller number of molecules due to a 5-fold reduction in the sensing area. Since the measurement of EOT through smaller arrays (with less holes) is straightforward, the process described here can be easily extended to the detection of sub-attomolar amounts of proteins. The inherent advantages of nanohole sensing relative to reflection SPR,[6] and the recent reports on inexpensive procedures for nanopatterning large areas,[17] indicates that these arrays are a very promising platform for future applications in multiplex biosensing.

Acknowledgment. We gratefully acknowledge funding support from NSERC, CFI, BCKDF, University of Victoria, Organization of American States, and the Brazilian agency, CAPES, and CNPQ (Project Nº 568802/2008-0).

Supporting Information Available: Experimental details for surface modification, SEM images, and CV experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Homola, J. *Chem. Rev.* **2008**, *108*, 462.
(2) Campbell, C. T.; Kim, G. *Biomaterials* **2007**, *28*, 2380.
(3) Genet, C.; Ebbesen, T. W. *Nature* **2007**, *445*, 39.
(4) Brolo, A. G.; Gordon, R.; Leathem, B.; Kavanagh, K. L. *Langmuir* **2004**, *20*, 4813.
(5) De Leebeeck, A.; Kumar, L. K. S.; de Lange, V.; Sinton, D.; Gordon, R.; Brolo, A. G. *Anal. Chem.* **2007**, *79*, 4094.
(6) Gordon, R.; Sinton, D.; Kavanagh, K. L.; Brolo, A. G. *Acc. Chem. Res.* **2008**, *41*, 1049.
(7) Ji, J.; O'Connell, J. G.; Carter, D. J. D.; Larson, D. N. *Anal. Chem.* **2008**, *80*, 2491.
(8) Lesuffleur, A.; Im, H.; Lindquist, N. C.; Lim, K. S.; Oh, S. H. *Opt. Express* **2008**, *16*, 219.
(9) Lesuffleur, A.; Im, H.; Lindquist, N. C.; Oh, S. H. *Appl. Phys. Lett.* **2007**, *90*, 261104.
(10) Stark, P. R. H.; Halleck, A. E.; Larson, D. N. *Methods* **2005**, *37*, 37.
(11) Tetz, K. A.; Pang, L.; Fainman, Y. *Opt. Lett.* **2006**, *31*, 1528.
(12) Szunerits, S.; Boukherroub, R. *Langmuir* **2006**, *22*, 1660.
(13) Lindquist, N. C.; Lesuffleur, A.; Oh, S. H. *Appl. Phys. Lett.* **2007**, 91.
(14) Marthandam, P.; Gordon, R. *Opt. Express* **2007**, *15*, 12995.
(15) Perez-Luna, V. H.; O'Brien, M. J.; Opperman, K. A.; Hampton, P. D.; Lopez, G. P.; Klumb, L. A.; Stayton, P. S. *J. Am. Chem. Soc.* **1999**, *121*, 6469.
(16) Enoch, S.; Popov, E.; Neviere, M.; Reinisch, R. *J. Opt. A* **2002**, *4*, S83.
(17) Henzie, J.; Lee, M. H.; Odom, T. W. *Nat. Nanotechnol.* **2007**, *2*, 549.

JA807704V