Surface layer reflective index changes of Au nanoparticle functionalized porous
silicon microcavity for DNA detection

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Abstract: A technique is demonstrated to detect DNA hybridization based on surface layer of Au/porous silicon microcavity (Au/PSM) substrate for very small amount of biomolecules. Simulations show that the increase of effective refractive index for the first layer of PSM will cause a blue shift for its reflectance spectrum and the blue shift becomes less with the increase of refractive index for one more layers. In experiments, such a blue shift of reflectance spectrum of PSM comes from the increase of refractive index by DNA hybridization on the surface. The detection limit of Au/PSM biosensor is 15.15 nM for 19-base pair DNA, which is comparable to that of reported biosensors based on porous silicon (PS). Therefore such an Au/PSM could be very useful to develop simple, rapid and sensitive optical biosensors when the amount of target is very small.

Key words: Porous silicon microcavity; Au nanoparticle; Reflectance spectrum; Blue shift; DNA biosensor.

1. Introduction

Porous silicon (PS) is a simple and efficient promising substrate for applications in drug delivery, early diagnostics and to be properly developed as a new effective miniaturized biosensor [1-5], owing to its large available surface area for molecular binding and its readily tunable pore structure formed into complex photonic crystal (PC) nanostructures, such as Bragg stacks, filters and microcavities [6-8]. In general, PCs have periodically modulated dielectric functions, resulting in light propagation dramatically different from the bulk material [9], so photonic devices are more compact and more sensitive than other optical devices and very suitable candidates for the realization of future passive and active optical devices [10-12]. Since the first demonstration of biosensors on the PS photonic crystal platform, many groups are working with different PC sensor to demonstrate higher sensitivity [13-16]. In fabrication and detection process of biosensor based on PS materials, many chemical modifications and functionalizations of PS surface for biosensors have been reported to improve sensitivity and stability, such as hydrosilylation, electrochemical alkylation and thermal hydrocarbonization, et al. [17-18]. However those methods show low flexibility in either the design of optical nanostructures or the control of pore size, and require the presence of reducing agents and stabilizers in contrast to common chemical methods. Especially in the fabrication and detection process of biosensor based on multilayer PS [14-16], when a small amount of chemical agents or biomolecule solution is dropped onto the prepared multilayer PS, such a solution probably can not penetrate uniformly into all layers and hence does not guarantee that the effective

refractive index of all layers changes the same, which will affect the spectral properties and the detection accuracy of biosensor based on multilayer PS.

Gold nanoparticles (AuNPs) seem to be the most versatile and extensively studied material among the various nanoparticles resulting from large surface area, good biocompatibility and excellent electron transfer. Since 1996, DNA-directed assembly has become one of the corner stones in bionanotechnology [19]. Among bio-applications based on AuNPs, detection of double stranded genomic DNA target is of remarkable significance. This is attributable to the discovery that thiols and other functional groups which have the capacity to strongly interact with AuNPs, which can be used to immobilize DNA strands on AuNPs [20-22]. The interplay effect in combining the AuNPs and PS is used in optical biosensor which is fully compatible for integrated circuit technology [23,24]. To our knowledge, there is rarely research on the biosensor based on single layer PS film with AuNPs [25]. Studies show that the change of the refractive index of PS will cause a reflectance spectra shift for Au/PS, but such a single PS film interference fringe is not easy to be distinguished and thus influences the sensitivity of this kind of biosensors, resulting from the absent of sharp resonance peaks.

To detect a small amount of target and improve the performance of biosensor based on Au/PS, we used one-dimension photonic structure to replace the single layer PS structure. Porous silicon microcavity (PSM) is of one-dimension photonic structure and can be obtained by inserting a Fabry-Perot cavity layer in between two symmetric Bragg reflectors. The interference of reflection of such two Braggs causes a high

reflectivity stop band with one narrow resonance peak approximately in its center, which means PSM has a high value of $Q (\lambda / \Delta\lambda)$ factor. Compared with single layer cases, the narrow spectral features of the resonant modes improve the resolution of spectral, which is a possible way for a low detection limit. Au/PSM biosensor was fabricated and used it for DNA detection by increasing refractive index only for the surface layer of PSM. Such a method needs neither chemical agent nor biomolecule to penetrate into more PS layers. The present research may provide an interesting way to develop a new optical label-free biosensor especially for a very small amount of biomolecule detection.

2. Experiments

2.1 Materials and Characterizations

3'-thiol modifier DNA oligonucleotide, complementary DNA oligonucleotide and non-complementary DNA oligonucleotide were purchased from Invitrogen (china, www.invitrogen.com). And the following DNA strands were synthesized for assay: probe DNA, 5'-TTGTACAGCAGCGTGCACC-(CH₂)₆-SH-3' (19-base), functionalized with alkane thiols at their 3' termini; Complementary DNA, GGTGCACGCTGCTGTACAA (19-base); and non-complementary DNA, ACACGTCATCGCTCTATTG (19-base).

Chloroauric acid (HAuCl₄·4H₂O₂, 48-50 % Au basis) was purchased from Aladdin Reagent Co. (China, www.aladdin-reagent.com). Phosphate buffer saline PH 7.4 (0.01 M PBS buffer solution) was obtained from Key Laboratory of Xinjiang Biological Resources and Gene Engineering.

Reflectance spectra were measured by UV-vis scanning spectrophotometer (Hitachi, U-4100, Japan) with wavelengths ranging from 400.00 to 2000.00 nm and a resolution 0.1 nm. Cross-sectional and surface images of Au/PSM substrate were obtained by a field-emission scanning electron microscope (Hitachi, S4800, Japan).

2.2 Preparation of Au/PSM Substrates

figure 1

Fig. 1 Schematic illustration of DNA functionalized Au/PSM biosensor fabrication. (a) Diagram and parameters of PSM structure. (b) AuNPs-based PSM substrate preparation. (c) Oxidized Au/PSM substrate. (d) DNA-functional AuNP/PSM self-assemble.

PSM is a one-dimensional photonic crystal constituted of a Fabry-Perot cavity in between two Bragg reflectors (DBRs). The reflectance maximum appears at Bragg wavelength (λ_0) with a relation: $m\lambda_0/2 = n_L d_L + n_H d_H$. The multilayer PS structure was constructed with low refractive index layers (n_L) and high refractive index layers (n_H) with effective optical thickness of $\lambda_0/4$ for each DBR layer and λ_0 for the central layer. Fig. 1a shows a schematic cross-section of the PSM following $(n_L n_H)^6 n_{Lc} (n_H n_L)^6$ sequence, where n_L represents high porosity or low refractive index and n_H represents low porosity or high refractive index. PS layers were fabricated by

electrochemical etching of a B-doped (100) Si substrate (resistivity 0.01 - 0.02 $\Omega\cdot\text{cm}$, thickness $400 \pm 10 \mu\text{m}$) in a mixed solution composed of 49% aqueous hydrofluoric acid and ethanol (volume ratio 1:1). The effective exposed area of the Si substrate was 0.785 cm^2 in teflon electrochemical etch cell and a copper was immersed into the electrolyte as a counter electrode. The periodic multilayer structure was fabricated by using a computer program (Labview) to alternately change current density for different etching times with a 5 s pause after each layer formation. The fabrication process begun from left to right and from top down into Si substrate following a sequence $(n_L n_H)^6 n_{LC} (n_H n_L)^6$, where for n_L the current density was 100 mA/cm^2 for 2.3 s, for n_H the current density was 40 mA/cm^2 for 3.6 s, and n_{LC} corresponds to microcavity layer with a current density of 100 mA/cm^2 for 9.2 s.

To form mental AuNPs, the fresh PSM was immersed into alcoholic metal salt solution which can uniformly wet the H-terminated hydrophobic PS surface. The metal salt was reduced by hydrogen, which results in metal nanoparticle formation and HCl synthesis during the reaction [26-27]. The process of AuNP deposition on PS layer can be categorized within redox potential of PS according to the following chemical equation:

The reduction reaction is

Dissolved Au from AuCl_4 behaves as an oxidizing agent, and the PS takes the role of reducing agent. These reactions favor the formation of the AuNPs. Furthermore,

AuNPs should deposit on SiO₂, and the structure of AuNPs-based fresh PSM substrate is shown in Fig. 1b.

To optimize Au deposition time, AuNPs were formed on the surface of the PSM by exposing fresh PSM to a 0.5 mM HAuCl₄ solution in ethanol for 5 min, 10 min, 15 min, and 20 min, separately. The freshly fabricated Au/PSM is unstable in the air due to the Si-H bond of fresh PSM easy to be oxidized. So passivation of the PS layer is necessary to achieve stable PS based devices. In order to ensure the stability of optical properties of Au/PSM substrate, all freshly fabricated Au/PSM substrates were soaked in H₂O₂ (30%) for 24 h at room temperature. Then all the substrates were rinsed with deionized water and dried in the air. After one month storage in room temperature, the Au/PSM substrates had not been aggregated and their spectroscopic reflectance properties were still stable, due to the existence of Si-O-Si, O-Si-H and O₃-Si-H groups [1-2, 14-16]. The preparation process of such a stable Au/PSM substrate is shown in step 2.

2.3 Functionalization of AuNPs

Probe DNA oligonucleotides were capped by thiol groups binding to AuNPs. When 30 μ l 50 μ M probe DNA were dropped onto the Au/PSM substrate at 37 °C for 2 h, AuNPs were functionalized with thiol-modified probe DNA, and then rinsed with PBS buffer solution three times in order to remove unbounded probe DNA and dried in the air. The structure of DNA-functional AuNP/PSM self-assemble is shown in Fig. 1d.

2.4 DNA Detections

In experiments, 15 μ l complementary DNA with different concentrations were

added onto probe DNA modified Au/PSM substrates for 2 h at 37 °C to ensure all target segments were recognized, then they were washed with PBS buffer solution three times to remove unhybridized complementary DNA and dried in the air. When the immobilized probe DNA in the pores of PS are exposed to complementary DNA, the two DNAs hybridization causes an increase of refractive index and thus causes a shift of the reflectance resonance peak.

3. Results and Discussions

In order to properly fabricate an Au/PSM biosensor, three main issues must be considered: appropriate structure of PSM to increase the sensitivity; appropriate assemble AuNPs in controllable size and shape, which is very important; and efficiency of biological molecule testing. Those three points will now be separately addressed.

3.1 Characterizations of Au/PSM

The size, shape and interparticle spatial properties of nanoparticles affect the unique nanoscale properties of nanomaterials. Impregnation of PSM in HAuCl_4 solutions is an effective method to synthesize AuNPs through a redox process involving Au^{3+} cations with a hydride covered surface. The spongiform PSM substrate supplies a large internal surface area of suitable nucleation sites for AuNP growth. Density and average size of AuNPs are strictly depended on both concentration of HAuCl_4 used for synthesis and deposition time. To study the effect of morphological parameters of AuNPs for the sensitivity of Au/PSM biosensors, we investigated the Au/PSM substrates under different deposition time with other parameters kept

unchanged. Fig. 2 shows plan-view FESEM images of the PSM substrate after being immersed into 0.5 mM HAuCl₄ solution for 5 min, 10 min, 15 min and 20 min separately. It can be clearly seen that surface pore dimension of PSM is 20 - 40 nm with random pore distribution, and the size of AuNPs dispersion increases with deposition time. In Fig. 2a, the average size of AuNPs on surface layer is 10 ± 4 nm with a not uniform AuNPs size distribution. In Fig. 2b, the average size of AuNPs is 13 ± 2 nm and the size distribution is uniform. Fig. 2c and Fig. 2d show that some of the sphere-like nanoparticles are combined with their neighbors into clusters which increase with deposition time. Meanwhile, smaller AuNPs (12 to 20 nm) fill in the gaps between larger AuNPs which are not uniform in size distribution.

figure 2

Fig. 2 Plan-view FESEM images of PSM substrate coated with AuNP deposition time for (a) 5 min, (b) 10 min, (c) 15 min and (d) 20 min.

figure 3

Fig. 3 (a) A cross-section FESEM image of Au/PSM substrate following $(n_L n_H)^6 n_{LC} (n_H n_L)^6$ sequence. (b) A detailed cross-section image of Au/PSM surface.

Fig.3 shows cross-sectional images of the PSM following $(n_L n_H)^6 n_{LC} (n_H n_L)^6$ sequence. In the process of electrochemical etching, higher anodization current gives rise to larger pores and hence larger porosity of PS layer. As shown in Fig. 3(a), the thickness of the PSM on silicon substrate is about 5.88 μm with a strong contrast between dark gray layer and light gray layer, which confirms that a large porosity difference has been achieved. The dark gray layers are high porosity PS layers or low refractive index (n_L) layers with thickness 220 nm, and the light gray layers are the low porosity PS layers or the high refractive index (n_H) layers with a thickness 195 nm. The thickness of low refractive index (n_{LC}) microcavity layer is about 900 nm. Fig. 3(b) shows the corresponding detailed cross-section of the PSM surface. The high porosity layer is composed of separated thin crystalline walls with a wire-like structure more isolated, while the low porosity layer is essentially uniform and the nanocrystals are more densely packed. It is important to note that AuNPs are formed on the surface of the PSM substrates. So AuNPs are functionalized with thiol modified DNA which

increases the effective refractive index of PSM surface.

3.2 Biological Detections of Au/PSM Biosensors

figure 4

Fig.4. Calculated reflectance spectra at 1393 nm of PSM structure following $(n_L n_H)^6 n_{Lc} (n_H n_L)^6$ sequence with refractive index n_L , n_H , and n_{Lc} to be 1.4, 1.7 and 1.4 respectively. (a) Effective refractive index on the surface of PSM increased by 0.3 for (1) 0 PS layer, (2) first PS layer and (3) top two PS layers; (b) Effective refractive index on the surface of PSM increased by 0.3 for (1) top four PS layers, (2) top six PS layers and (3) top eight PS layers; (c) Effective refractive index of the first PS layer increased by (1) 0, (2) 0.3 and (3) 0.6; (d) Experimentally measured reflectance spectra with Au deposition time for (1) 0 min, (2) 5 min, (3) 10 min, (4) 15 min and (5) 20 min.

It is well-known that when a multilayer PS is exposed to chemical or biological molecules, the effective refractive index of each layer increases to make overall

reflectivity spectrum shift to higher wavelength, known as red shift. This method is used to detect and identify unknown chemical or biological molecules in most of sensing applications based on PS. In the processes of oxidization, silanization, immobilization and detection, it must ensure that chemical agents and biomolecules react with all PS layers completely. In the above general process for the fabrication and the detection of biosensor, it is usually assumed that all these processes are ideal which means the increase of effective refractive index for every layer is the same. However, in experiments, especially in immobilization or detection, a small amount of solution dropped onto the multilayer PS substrate could not guarantee that the effective refractive index of all PS layers changes the same, which will influence the sensitivity and the accuracy. The reflectance spectra of PSM shifts with respects to permeable layer numbers of PSM and the experimental data are given in supplementary materials (Fig. 1s).

Under above considerations, to verify detection mechanism of the PSM as an optical sensor, we presented several simulations to monitor reflectance spectrum changes of PSM by using the transfer matrix method. Theoretical results show that there exists a narrow resonance peak at 1393 nm in the reflectance spectrum of PSM with $(n_L n_H)^6 n_{Lc} (n_H n_L)^6$ sequence. Fig. 4a shows three reflectance spectra for the cases when the effective refractive index increases by 0.3 for 0 layer, for the first layer, or for top two layers. The reflectance spectra of the PSM respectively shift to 1393 nm, 1386 nm or 1389 nm corresponding to those three cases. Therefore, the increase of effective refractive index only for the first layer of PSM causes a blue shift, and the blue shift of

the reflectance spectrum becomes less when one more layer refractive index increases. The reflectance spectrum of PSM begins to shift to a higher wavelength (red shift) when enough top layer's refractive indices increase by the same value. In Fig. 4b, effective refractive index for top four layers, top six layers or top eight layers were increased by 0.3, and the corresponding resonance peaks shift to 1393 nm, 1401 nm and 1417 nm respectively. So the more top layers of the refractive index increased there are, the more red shift there will be. In detection process, when test biomolecules enter into only few layers of the PSM, the effect of the refractive index change for the first layer of PSM can not be neglected, and the blue shift effect should be taken into account when spectral properties of PSM are analyzed.

We fabricated PSM biosensor trying to detect the blue shift of reflectance spectrum by increasing effective refractive index only for the surface layer. Fig. 4c shows the reflectance spectrum with the resonance peak located at 1387 nm and 1382 nm from simulations, where the effective refractive index for the first layer was increased by 0.3 and 0.6 respectively. The reflectance spectrum shifts to lower wavelength (blue shift) with the increase of the reflective index of the first layer. Fig. 4d shows experimentally measured reflectance spectra with 0 min, 5 min, 10 min, 15 min and 20 min Au deposition time for fresh PSM separately. With Au deposition time increasing, the effective refractive index of the first layer of PSM increases and the reflectance spectrum moves to lower wavelength (blue shift). The blue shifts of the reflectance spectra of Au/PSM for all those cases of different deposition time indicate that AuNPs was formed only on the surface of the PSM (see Fig.2 and Fig.3), which

are in good agreement with our simulated reflectivity discussed in the previous section and shown in Fig. 4c. Meanwhile, with Au deposition time increasing, the size of AuNPs becomes larger and less uniform in distribution, and the narrow spectral features of the resonant mode will be destroyed. Reflectivity of light for Au/PSM is decreased compared with that of non-treated PSM, due to the absorption induced by metallic nanoparticles.

3.3 Evaluations of Conjugation Efficiency and Specific Selectivity

figure 5

Fig.5. (a) Reflectance spectra of fresh PSM after each functionalization step: Au deposition, Au/PSM oxidation and 50 μ M probe DNA binding to AuNPs. (b) Blue shifts of resonance peak for Au deposition times of 5 min, 10 min, 15 min and 20 min separately for 6 μ M complementary DNA detection. (c) Negligible shift of resonance peak of Au/PSM biosensor for non-complementary DNA. (d) Blue shift of resonance peak of Au/PSM biosensor for complementary DNA, demonstrating recognition of

DNA binding to the AuNPs on the surface of PSM.

UV-vis spectrophotometer was used to study the change of reflectance spectra of the Au/PSM substrate before and after probe DNA binding to AuNPs. Fig. 5a shows the reflectance spectra for each step of probe DNA functionalization (from right to left): after AuNPs formation, after oxidation process and after probe DNA binding to AuNPs. After PSM being oxidized, there is an approximately 110.0 nm blue shift of reflectance spectrum, since the effective refractive index of silicon dioxide is less than silicon. An approximately 30 nm blue shift of the reflectance resonance peak can be observed after the oxidized Au/PS substrate being exposed to 50.0 μ M probe DNA. This suggests that the 3'-thiol modifier DNA oligonucleotide bind well to AuNPs on the surface of PSM, which increases the effective refractive index only for the first layer. In order to establish correlations between the morphology of AuNPs on the surface of PSM and efficient binding of probe DNA with AuNPs, the PSM substrate was separately immersed into 0.5 mM HAuCl₄ solution for 5 min, 10 min, 15 min and 20 min separately for 6 μ M complementary DNA detection. The blue shifts of resonance peaks corresponding to those deposition times are 9.00 nm, 14.33 nm, 7.33 nm and 5.00 nm, respectively, and the detailed data are given in supplementary materials (Fig. 2s and Tab. 1s). It can be seen from Fig. 5b that 10 min is the best deposition time, since it corresponds to the maximum blue shift, and AuNPs on PSM are homogeneously formed with 12-15 nm in size in this case (in Fig. 2b). In order to verify the feasibility of this oxidized Au/PSM substrate as an optical biosensor, controlled experiments were performed on the oxidized Au/PSM substrate to demonstrate selectivity. Fig. 5c shows

the reflectance resonance peak of Au/PSM substrate after exposing to 50.0 μM non-complementary DNA and there is a negligible shift. This is because the probe DNA and non-complementary DNA do not bind, so the refractive index of the Au/PSM keeps unchanged. In order to enable specific detection of complementary DNA oligonucleotide, the complementary DNA was used to target DNA with Au/PSM biosensor. Fig. 5d shows that the blue shift of reflectance resonance peak is 61.0 nm after the Au/PSM was exposed to 10.0 μM complementary DNA. This can be explained by the fact that the probe DNA and the complementary DNA hybridization increase the effective refractive index of the first layer of PSM. The blue shift of reflectance resonance peak suggests that the oxidized Au/PSM substrate has been carried out successfully for specific detection which is able to select DNA hybridization between complementary and non-complementary DNA.

3.4 Target DNA Detections

figure 6

Fig. 6 Blue shifts of resonance peaks based on DNA sensor after PSM being exposed to different concentration of DNA from 2.0 μM to 50.0 μM . The inset shows the fitted

linear analysis for complementary DNA concentration ranges from 2.0 μM to 10.0 μM and the error bars show the standard deviations of three repeated measurements for three different Au/PSM biosensors.

The blue shift of reflectance resonance peak quantifies DNA concentration, and a larger shift corresponds to a higher concentration. Fig. 6 shows a blue shift of reflectance resonance peak as a function of concentration (i.e. 0.0 μM , 2.0 μM , 4.0 μM , 6.0 μM , 8.0 μM , 10.0 μM , 12.5 μM , 25.0 μM , 50.0 μM) for 19-base pair complementary DNA and all the reflectance spectra in supplementary materials (Fig. 3s). When the Au/PSM biosensors were exposed to these complementary DNA, probe DNA and complementary DNA bind on the surface of PSM with AuNPs as cross-linkers. The effective refractive index of the first layer of PSM causes a blue shift. The blue shifts corresponding to those concentrations of complementary DNA are 2.0 nm, 9.0 nm, 13.0 nm, 23.0 nm, 42.0 nm, 61.0 nm, 28.0 nm, 24.0 nm and 22.0 nm respectively. The value of blue shift is strongly correlated with the amount of complementary DNA captured by the Au/PSM biosensor. We can see the linear fit of the experimental data in inset of Fig. 6. The linearity of the blue shift of the reflectance spectrum as a function of concentration of complementary DNA is in a range from 2.0 μM to 10.0 μM . The linear equation is $y = -9.8 + 6.6 C$ and the correlation coefficient is 0.969, where y represents the blue shift of the resonance peak and C represents the concentration of complementary DNA. As the resolution of UV-vis spectrophotometer is 0.1 nm, the lowest detection limit of such biosensor is $0.1/6.6 = 15.15$ nM. The reproducibility of the Au/PSM biosensor had been undertaken in triplicate for three

samples under same condition. The DNA detection limit based on Au/PSM biosensor is of nM concentration, which is comparable to that reported for DNA detection by means of the change of effective refractive index, or red shift, such as PSM biosensor based on SOI substrate for detecting 19-base pair DNA with a limit of 43.9 nM [15], multilayer PS polybasic symmetric structure for 23-base pair DNA detection with a limit of 21.3 nM [16] and PS waveguide for 24-base pair DNA detection with a limit of 50.0 nM [14]. Furthermore, compared to other biosensors based on PS, such an Au/PSM biosensor has the advantage in that there needs only a small amount of chemical agents or biomolecule solution, even if it can not penetrate uniformly into all layers. The fabrication of such an Au/PSM biosensor is simpler, chemical agents are fewer and target DNA are much less needed for detection.

4. Conclusions

Au/PSM biosensor was firstly fabricated with AuNPs as cross-linker to link PSM substrate and probe DNA. Based on simulations, the increase of effective refractive index for the first layer of PSM will cause a blue shift for the reflectance spectrum of PSM, and the blue shift becomes less with the increase of refractive index for one more layers of the PSM. There will be a red shift when it goes deeper enough. In experiments, Au/PSM substrate was fabricated by fresh PSM being immersed into HAuCl_4 solution. Au deposition time was optimized to achieve a maximal blue shift of reflectance spectrum for probe DNA functionalization. The blue shift due to the increase of refractive index of the first layer through DNA hybridization on the surface of PSM substrate means that the surface layer reflective index changes can be used as

a biosensor. The detection limit of Au/PSM is 15.15 nM for 19-base pair DNA detection, which is comparable to that of reported biosensors based on PS [14-16]. This method could be very useful in the development of simple, rapid and sensitive optical biosensors based on Au/PSM substrates when biomolecules cannot fully enter into PSM, and has an advantage for the cases when the amount of target is very small.

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