A label-free single photonic quantum well biosensor based on porous silicon for DNA detection*

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The single photonic quantum well (PQW) structures are successfully fabricated on p-type silicon wafer by electrochemical etching process, and are used for DNA detection firstly. The red shift of resonance peak is caused by the changing refractive index of PSi layer, which results from coupling of organic molecules into pores. When the porous silicon (PSi) based single PQW biosensors are immersed in complementary deoxyribonucleic acid (DNA) with different concentrations ranging from 0.625 μM to 10.000 μM, a good linear relationship is observed between the red shift of resonance peak and the complementary DNA concentration. Experimental results show that the detection sensitivity of PSi-based single PQW biosensors is 3.04 nm/μM with a detection limit of 32 nM for 16-base pair DNA oligonucleotides.

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Since the discovery of visible luminescence from porous silicon (PSi) emission at room temperature was reported in 1990[1], a great number of researches have been conducted about the structural improvement, the optical properties and application, etc[2-4]. PSi possesses the large specific internal surface area up to 1000 m^2/cm^3 and the size-selective penetration characteristics. These interesting properties make PSi an almost ideal host material for sensor applications[5-7]. The large specific internal surface area of PSi can enable large amounts of biomolecules to interact in a small working area, and results in a high sensitivity. The size-selective penetration characteristics can make PSi meet biomolecule detection with different sizes. Therefore, biosensors based on PSi material attract intense research, especially label-free optical biosensor[8-12].

Biomolecular detection plays a very important role in every field of our lives, such as healthcare, food safety and environment monitoring[13,14]. So the biosensor market demand is more and more high. Especially, biosensors with higher sensitivity are needed for detection of lower concentration. In order to address these challenges, much of work has been done to find new methods which can improve sensitivity and reusable traits of biosensor. Various label-free optical biosensors based on PSi configurations were proposed[15-18], such as single layer Fabry-Pérot cavity, Bragg mirror, waveguide and microcavity (MC), and high performance detection platform by improving the preparation conditions.

Photonic quantum well (PQW) structure was proposed in 1994 for the first time[19], and it has been investigated both experimentally[20] and theoretically[21]. PQW structure can be constructed by combining different photonic crystals and sandwiching a well between two barriers.

In this paper, the single PQW structures based on PSi are obtained by electrochemical etching process. The coupling of organic molecules into the pores causes changes in the refractive index of the medium, which consequently induces a shift of resonance peak. Therefore, the single PQW structure based on PSi can serve as a sensitive platform for biomolecular detection. So, for the first time, we successfully produce a label-free single PQW biosensor based on PSi. It is of low cost, fast response and easy preparation.

Fig.1(a) shows a schematic diagram of the current density applied in the experimental electrochemical etching process, and Fig.1(b) shows the cross-section view of PQW structure. PQW structure $(AB)_m(CD)_n(AB)_m$ is formed by combining two different photonic crystal sequences $(AB)_m$ and $(CD)_n$, where m and n are the numbers of periods of photonic crystal, respectively. It is similar to the semi-

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conductor quantum well structure composed of potential barrier and well. As shown in Fig.1(b), the photonic crystal $(AB)_m$ plays a role of potential barrier and $(CD)_n$ corresponds to the potential well. Photonic crystal is a dielectric material whose refractive index distributes periodically, which produces a periodic potential field in photonic crystal to form the photonic band gap (PBG) structure. For these reasons, the movement of photons is restricted. The confinement effect results in the frequency quantization. Each quantized frequency corresponds to the different bound states of the PQW structure. The bound state is the discrete conduction band of well. The number of bound states equals the period of photonic crystal well $(CD)_n$, and the transmission rate of each bound state is close to 1.

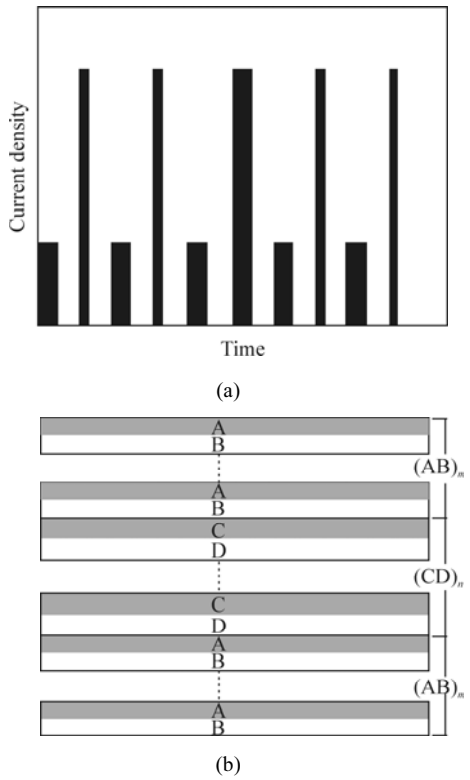


Fig.1 (a) Current density applied in the electrochemical etching experiment; (b) Cross-section view of PQW structure

The electromagnetic waves can propagate through PQW structure without any attenuation by resonant tunneling, that is to say transmission rate is 1, only when the incident photon energy is consistent with the energy of PQW structure bound state. Tunneling probability is almost 0 when they are not equal. The phenomena indicate that we can obtain the desired transmission peak frequency by adjusting the parameters of barrier and well, such as the thickness and dielectric constant.

The single PQW structures of $(AB)_5(CD)_1(AB)_5$ were prepared. All samples were formed by electrochemical etching of p-type <100> silicon wafers (resistivity of 0.01－0.02 Ω·cm, 420±10 μm thickness) in electrolyte solution of 49% aqueous hydrofluoric acid and absolute ethanol in a volumetric ratio of 1:1. Before electrochemical etching, acetone, ethanol and deionized (DI) water were used for cleaning the surface of monocrystalline silicon with ultrasonic bath respectively for 20 min. The electrochemical etching process of all samples was computer-controlled with the LabView platform. A and B layers of distributed Bragg reflector (DBR) structure were fabricated with an etching current density of 20 mA/cm^2 for 4 s and 80 mA/cm^2 for 3 s, and C and D layers of middle photonic crystal well structure were fabricated at 20 mA/cm^2 for 7.6 s and 80 mA/cm^2 for 4.5 s. After electrochemical etching, all samples were rinsed with DI water and dried in ambient air at room temperature. In order to increase pore diameter, each piece of sample was soaked in 20 mL of 1.5 mM KOH containing ethanol solution for 30 min[22].

Since the optical performance of freshing etched PSi structure is unstable, all samples must be oxidized with H_2O_2 solution for 24 h at room temperature to obtain Si-OH, Si=O and Si-O-Si groups. Then in the silanization step, samples were immersed in 20 mL 5% 3-aminopropyltriethoxysilane (APTES) solution, composed of 10 mL 99% 3-APTES (Aladdin), 10 mL DI water and 10 mL methanol, for 1 h. Then samples were rinsed with DI water and placed in the environment of 100 ℃ for 10 min. Silanization treatment is to obtain the covalent attachment of reactive groups of silane. In the final step, samples were immersed in 2.5% glutaraldehyde solution for 1 h, and then rinsed three times by using phosphate buffer silane (PBS, PH: 7.4) to clean excess glutaraldehyde. Glutaraldehyde molecules were used as the coupling agent between silane groups and biomolecules. Above functionalization steps are necessary for following biomolecule immobilization and detection.

Three DNA oligonucleotides were prepared, and the sequences are given as follows:

Probe DNA: 5′-TACAGCAGCGTGCACC-3′ (16-base),

Complementary DNA: 5′-GGTGCACGCTGCTGTA-3′ (16-base),

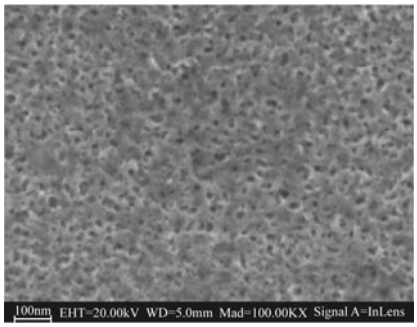
Non-complementary DNA: 5′-ACACGTCATCGCTC -TA-3′ (16-base).

Every PSi-based single PQW biosensor was exposed in 50 μL probe DNA solution for 2 h at 37 ℃ incubator, and then rinsed with PBS buffer to remove excess DNA. The samples were dipped into 3M EA (>99% ethanolamine hydrochloride) at 37 ℃ incubator, laid for 2 h, and then rinsed with PBS buffer and dried at room temperature. The last step was to make PSi-based single PQW biosensors exposed to complementary DNA with different concentrations for 1.5 h at 37 ℃ incubator, which were rinsed using PBS buffer and dried in the air.

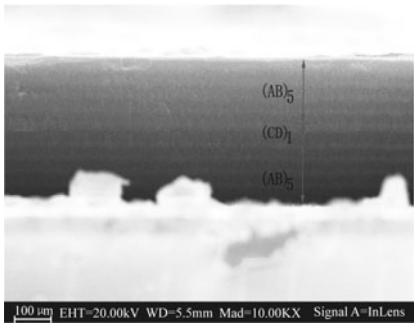
The top view and the cross-section image of the surface of PSi-based single PQW biosensor were obtained by using ZEISS SUPRA 55VP (Zeiss SMT, Germany) field scan electron microscope (FSEM). The reflectance spectra of samples were measured with ultraviolet-visi-

ble (UV-vis) spectrophotometer (Hitachi U-4100, Japan).

Fig.2 clearly demonstrates that the average pore diameter is approximately 20 nm, which is big enough to make effective infiltration for biomolecules. In Fig.2(b) the dark gray stripes correspond to PSi layers with high porosity (low refractive index), whereas the light gray stripes correspond to PSi layers with low porosity (high refractive index).



(a) Top view



(b) Cross section

Fig.2 Top view and cross section of the PSi-based single PQW biosensor

Fig.3 shows the reflectance spectra of PSi-based single PQW structure after functionalization treatment. It shows an obvious red shift after the APTES and glutaraldehyde, which is because of the coupling of small organic molecules and results in an increase of the refractive index of PSi layers. The red-shift phenomenon denotes that the functionalization treatment of samples is successful, and it is essential for following DNA biomolecular immobilization and detection. Fig.4(a) shows that there is a red-shift of 47 nm in reflectance spectra. It results from the specific binding of probe DNA to complementary DNA when PSi-based single PQW biosensor is immersed in 50 μM complementary DNA. The specificity binding causes the increase of effective refractive index, and then produces a red shift. In order to account for the specificity, control experiments are conducted as follows: one PSi-based single PQW biosensor is immersed in 50 μM non-complementary DNA, and another is immersed in PBS buffer. Experiment results show a negligible shift in reflectance spectra as shown in Fig.4(b) and (c). Therefore, the red shift of reflectance spectra is owing to the selective DNA hybridization.

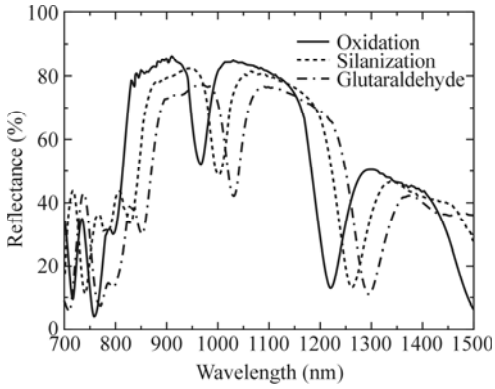
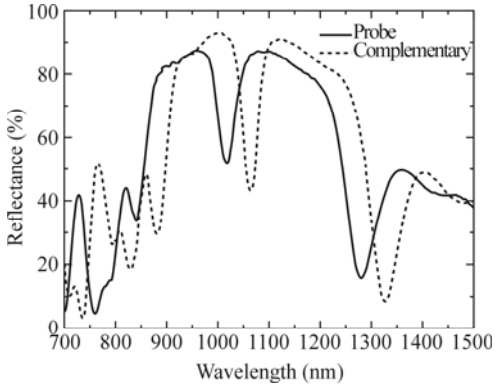
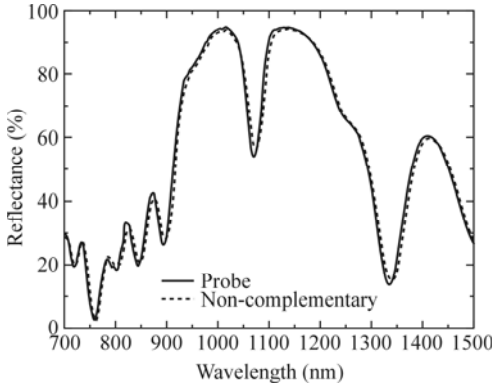


Fig.3 Reflectance spectra of PSi-based single PQW structure after oxidation, silanization and glutaraldehyde treatments

The complementary DNA was diluted into different concentrations. When PSi-based single PQW biosensors were immersed in complementary DNA with different concentrations, the selective DNA hybridization causes an increase of effective refractive index of PSi layer, and thus produces a red shift of resonance peak. As shown in Fig.5, the red shift of experimental reflectance spectra as a function of the concentration of complementary DNA is given. The red shifts of resonance peak are 3.0 nm, 6.5 nm, 9.2 nm, 18.0 nm and 32.0 nm, corresponding to different complementary DNA concentrations of 0.625 μM, 1.250 μM, 2.500 μM, 5.000 μM and 10.000 μM, and a good linear relationship is observed. The experimental



(a)



(b)

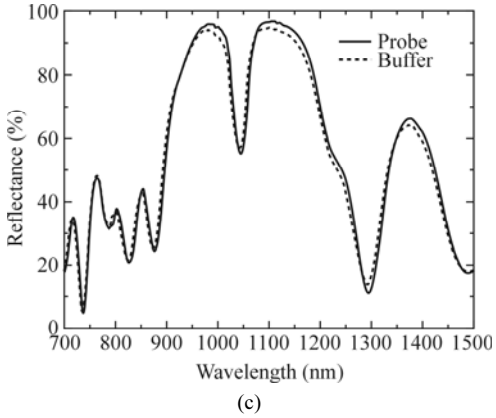


Fig.4 Reflectance spectra of PSi-based single PQW biosensor before and after soaking in (a) 50 μM complementary DNA, (b) 50 μM non-complementary DNA and (c) buffer solution

data show the sensitivity of sensor is 3.04 nm/μM. The lowest detection limit is 0.1 nm/3.04 μM=32 nM, where 0.1 nm is resolution of the device. And we make repeatability experiment that there are two PSi-based single PQW biosensors obtained under the same preparation conditions, which were immersed in every complementary DNA with different concentrations. Results show that the reproducibility of the PSi-based single PQW biosensor is good.

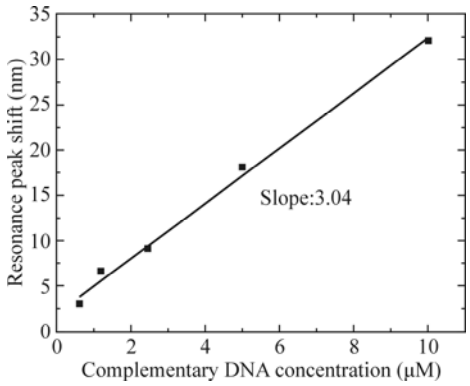


Fig.5 Red shift of resonance peak as a function of concentration of complementary DNA

We successfully prepared a single PQW structure based on PSi by electrochemical etching process, and used it for DNA detection for the first time. The coupling of organic molecules into the nanoscale pores causes the changes in the refractive index of PSi layers, which induces a shift of resonance peak to long wavelength. Experimental results show that the red shift of resonance peak is linear with the complementary DNA concentration ranging from 0.625 μM to 10 μM. The detection sensitivity is 3.04 nm/μM with a detection limit of 32 nM for 16-base pair DNA oligonucleotides. Because of relatively high sensitivity, convenient fabrication, low cost, fast response and good reproducibility, the PSi-based single PQW biosensor is promising in DNA detection. In addition, the work also provides a simple and sensitive platform for other biomolecules detection.

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