# A localized surface plasmon resonance DNA biosensor based on gold nanospheres coated on the tip of the fiber*

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A localized surface plasmon resonance (LSPR) biosensor was prepared with gold nanospheres (AuNSs) coated on the tip face of the optical silica fiber. AuNSs with the sizes of 20 nm and 80 nm were used. The sensitivities of $AuNS_{20\ nm}$ and $AuNS_{80\ nm}$ modified sensors to bulk refractive index (RI) variation are 82.86 nm/RIU and 218.98 nm/RIU, respectively. The $AuNS_{80\ nm}$ modified sensor was used for the detection of 40 bases DNA hybridization and the limit of detection is 50 nmol/L, where the 40-bases DNA probe was covalently linked with $AuNS_{80\ nm}$. The complementary DNA sequence in tris-acetate-EDTA (TAE) buffer solution was detected as the target DNA. This fiber sensor has the advantages of small sample consumption, easy fabrication and high sensitivity.

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Localized surface plasmon resonance (LSPR) is a resonance phenomenon of collective electron charge oscillations in metal nanoparticles[1,2]. LSPR sensors have been applied to a wide range of biochemical interactions[3,4]. Most of the substrates for LSPR sensors are silica glasses. Recently, optical fiber has been used as the substrate, because it has the advantages of simple structure, easy integration, remote measurement and high sensitivity. The main configurations of optical fiber LSPR sensors include transmission-based fiber sensor[5] and reflection-based fiber sensor[6,7] with AuNSs modified on the side wall of the optical fiber. The fiber sensor with AuNSs on its tip has the advantages of less sample consumption, easier preparation, and much smaller sensing region than the above two configurations[8]. This paper describes a fiber biosensor for DNA detection modified with AuNSs and thiolated ss-DNA as detecting probe on the tip of fiber. The complementary DNA sequence was detected as the target DNA. The wavelength-based detection mode instead of intensity-based mode was used to avoid the influence of some interference.

Multimode optical fibers with diameter of 600 μm and numerical aperture (*NA*) of 0.37 were bought from Thorlabs. The replaceable optical fiber sensor was affixed onto the end of a bifurcated optical fiber. The white light from a DT-MINI-2-GS light source (Ocean Optics) was guided into the sensor probe. The reflected light was fed into a compact CCD spectrometer with a detection spectral range from 200 nm to 980 nm (Ocean Optics, QE65pro, *SNR*=1 000:1) (see Fig.1) and produced the typical LSPR spectra. Each recorded spectrum was averaged for 3 times and smoothed for 10 times to reduce the noise. The LSPR spectra were smoothed by Savitzky-Golay method using Origin 8.5. The LSPR peak positions were calculated by weighted centroid method using Matlab R2007a.

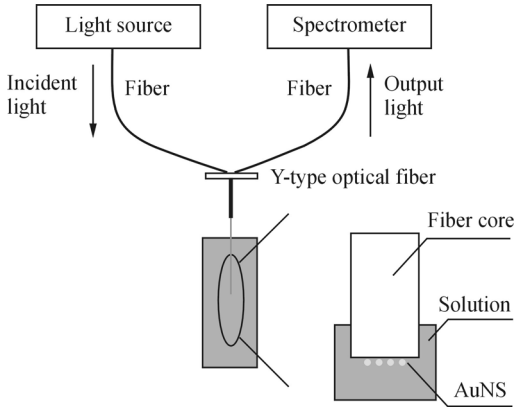


**Fig.1 Schematic diagram of the detection system and AuNS-modified optical fiber sensor**

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The chemical reagents of poly(allylamine hydrochloride) (PAH) and mercaptohexanol were bought from Sigma-Aldrich. NaCl, glycerol, ethanol, $H_2SO_4$ (98%) and $H_2O_2$ (30%) were bought from Sinopharm Chemical Reagent Beijing Co., Ltd. The ss-DNA sequence (probe) (5'-SH-$(CH_2)_6$-AAAACACACAGGGAAGGGAAGGGACACA-CAAAAGCCAAAA -3') and the complementary strand sequence (target DNA sequence) (5'-TTTTGGCTTT-TGTGTGTCCCTTCCCTTCCCTGTGTGTTTT-3') were synthesized by Shanghai Sangon Biotechnology Co., Ltd. All the reagents and chemicals are analytical grade. All procedures were operated at room temperature if not specially illustrated. Deionized (DI) water (18 mΩ) used in experiments was deionized by Millipore.

A 10 cm optical fiber was taken and 2 cm of its cladding was removed at both ends by stripping tool. Both ends of the fiber should be polished carefully by emery papers with 10 μm, 3 μm, 1 μm roughness and ADS in sequential order. Then one end face of the fiber was dipped into a piranha solution consisting of 30% $H_2O_2$ and concentrated $H_2SO_4$ (volume ratio is 3:7) for 1 h at 65 °C. After a thorough rinse with DI water, the end face was functionalized with PAH solution (2 mg/mL, 1 mol/L NaCl) for 30 min at room temperature. PAH was a positively charged polyelectrolyte. It was adsorpted on the hydorxyl silica surface by electrostatic force. After thoroughly rinsing with DI water, the end face of fiber was immersed into the AuNS colloid solutions (citrate capped, TedPella) for 1 h to form a self-assembled AuNS monolayer on the end face of the silica core. The fiber sensor modified with AuNSs was then immersed into the 0.05 mol/L TAE (pH=8) for 30 min. After a stable LSPR baseline was observed, the fiber sensor was immersed into 1 μmol/L thiolated ss-DNA probe in 0.05 mol/L TAE (pH=8) solution for about 2 h to incubate the formation of the covalent bond between the thiolated ss-DNA and AuNSs. The fiber tip was rinsed excessively by 0.05 mol/L TAE (pH=8) and then was immersed into 1 mmol/L mercaptohexanol ethanol solution for about 2 h.The sensor was then rinsed with copious quantities of DI water. The hybridization procedure was measured by this thiolated ss-DNA probe modified fiber sensor.

$AuNS_{20\ nm}$ and $AuNS_{80\ nm}$ were immobilized on the two fibers by electrostatic self assembly respectively. The SEM images (S-4800 SEM, HATACHI) for AuNS monolayers are shown in Fig.2. The surface density of $AuNS_{20\ nm}$ is higher than that of $AuNS_{80\ nm}$, because of the easier adsorption for $AuNS_{20\ nm}$. Both of $AuNS_{20\ nm}$ and $AuNS_{80\ nm}$ have relatively uniform distribution and no aggregation.

In this study, the fiber sensor was dipped into glycerol solutions with different concentrations ranging from 0% to 100% to determine the RI sensitivity. The relationship between concentrations of glycerol solutions and their refractive indices which were tested by Abbe refractometer at 25 °C is shown in Tab.1. The optical fiber sensor was dipped into the glycerol solutions from low RI to high RI and kept standing for 5 min in each solution. Fig.3 shows absorbance spectra of the fiber sensor for different RI values of the surrounding medium. The bulk RI sensitivity can be expressed by the shift of the resonance peak per refractive index unit (RIU)[9] as

$$S = \frac{\delta\lambda}{\delta n}, \tag{1}$$

where $S$ is the sensitivity of the bulk refractive index, $\lambda$ is the wavelength of the resonance peak, and $n$ is the refractive index of the medium.

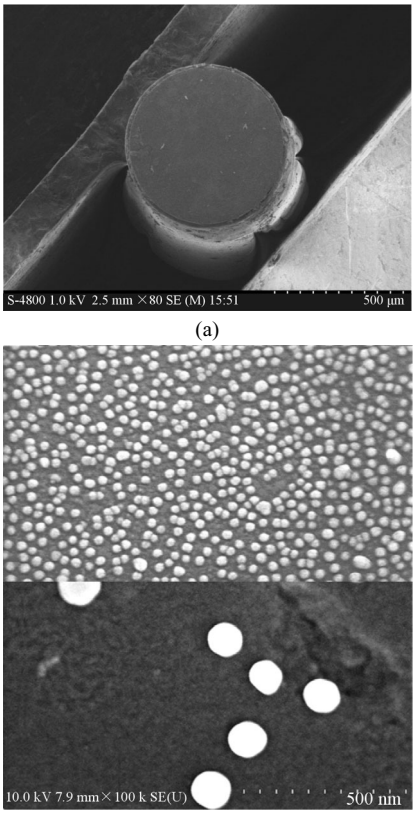


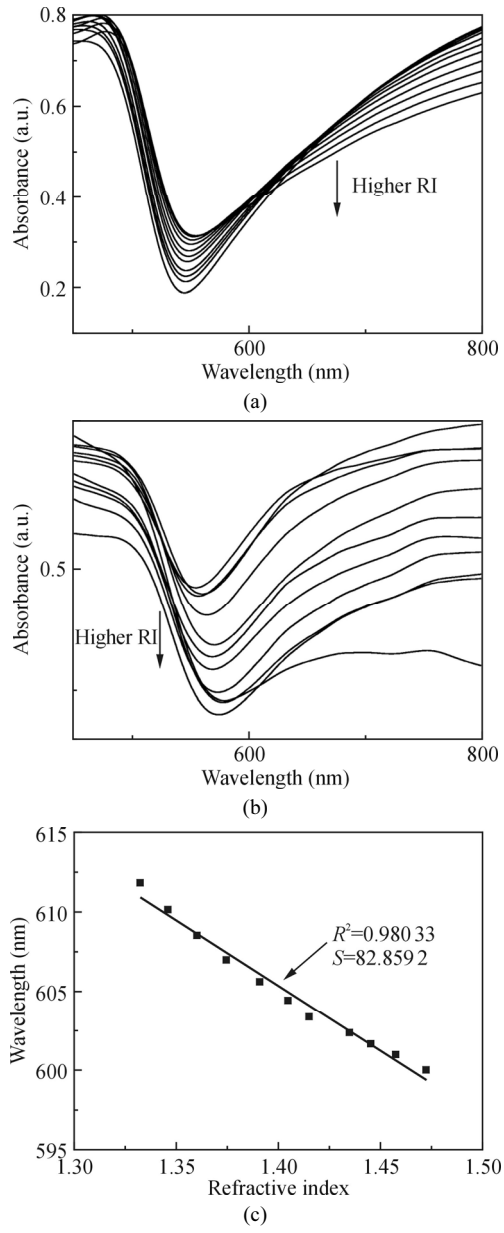

**Fig.2 The SEM images: (a) The tip of the fiber sensor; (b) Electrostatic self-assembly monolayers of $AuNS_{20\ nm}$ (upper) and $AuNS_{80\ nm}$ (lower)**

**Tab.1 The refractive indices corresponding to the concentrations of glycerol solution**

| Sample | The concentration of glycerol solution | Refractive index |
|---|---|---|
| 1# | 0% | 1.332 4 |
| 2# | 10% | 1.345 5 |
| 3# | 20% | 1.360 0 |
| 4# | 30% | 1.374 4 |
| 5# | 40% | 1.391 1 |
| 6# | 50% | 1.404 5 |
| 7# | 60% | 1.415 1 |
| 8# | 70% | 1.434 9 |
| 9# | 80% | 1.444 9 |
| 10# | 90% | 1.457 5 |
| 11# | 100% | 1.471 9 |

The absorbance spectra of $AuNS_{20\,nm}$ modified fiber sensor for glycerol solutions are shown in Fig.3(a). The curves display a blue shift with the increase of the refractive index. The sensitivity is about 82.86 nm/RIU ($R^2$=0.980 33) in Fig.3(c). In Fig.3(b), the absorbance spectra of $AuNS_{80\,nm}$ modified fiber sensor have a red shift with the increase of the refractive index. The sensitivity is about 218.98 nm/RIU ($R^2$=0.996 31) in Fig.3(d).

The $AuNS_{80\,nm}$ coated fiber sensor was used as the DNA biosensor because of its higher sensitivity. In order to detect a certain sequence target DNA by using this optical fiber sensor, a complementary thiolated ss-DNA probe was bonded with $AuNS_{80\,nm}$ by Au-S bonds at room temperature. With the thiolated ss-DNA probe immobilized onto the $AuNS_{80\,nm}$, a dense shell was formed which increased the RI at the surface of AuNSs. The thiolated ss-DNA probes immobilized onto $AuNS_{80\,nm}$ are disorderly. The fiber



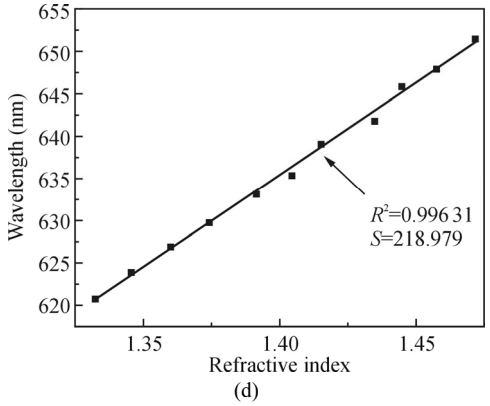




**Fig.3 The absorbance spectra of sensors modified with (a) $AuNS_{20\,nm}$ and (b) $AuNS_{80\,nm}$ for surrounding RI ranging from 1.332 4 to 1.471 9; Plots of RI sensitivities of sensors modified with (c) $AuNS_{20\,nm}$ and (d) $AuNS_{80\,nm}$**

sensor modified by thiolated ss-DNA probes was dipped into 1 mmol/L mercaptohexanol ethanol solution to promote the adsorption of mercaptohexanol and the displacement of thiolated ss-DNA probes. Then the thiolated ss-DNA probes became a well-aligned DNA monolayer. The capability of the ssDNA functionalized LSPR fiber sensor was evaluated by hybridization assay[10]. The target DNA was hybridized with probe DNA in certain conditions on the basis of DNA hybridization principle (Fig.4). In this study, the hybridizating condition was about 0.05 mol/L TAE solution (1 mol/L NaCl, pH=8) at 60 °C.

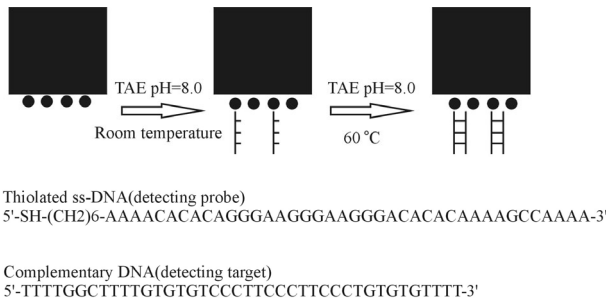



**Fig.4 The schematic diagram of adsorption process for thiolated ss-DNA and complementary DNA**

Target DNA solutions with the concentration ranging from 0 to 500 nmol/L were measured. The wavelength shifts of LSPR peaks with different concentrations are investigated to determine the detectability of the target DNA. Fig.5(a) shows that the LSPR peak has a red shift with the concentration increasing from 0 to 500 nmol/L. Fig.5(b) shows that the shift of wavelengh increases from 543.91 nm to 546.46 nm with the concentration increasing from 0 to 500 nmol/L. And as low as 50 nmol/L target DNA can be detected.

In this study, we designed and fabricated an LSPR sensor with AuNSs coated on the end face of the optical fiber. We study the influence of the AuNS size and come to a conclusion that the $AuNS_{80\,nm}$-modified sensor has a

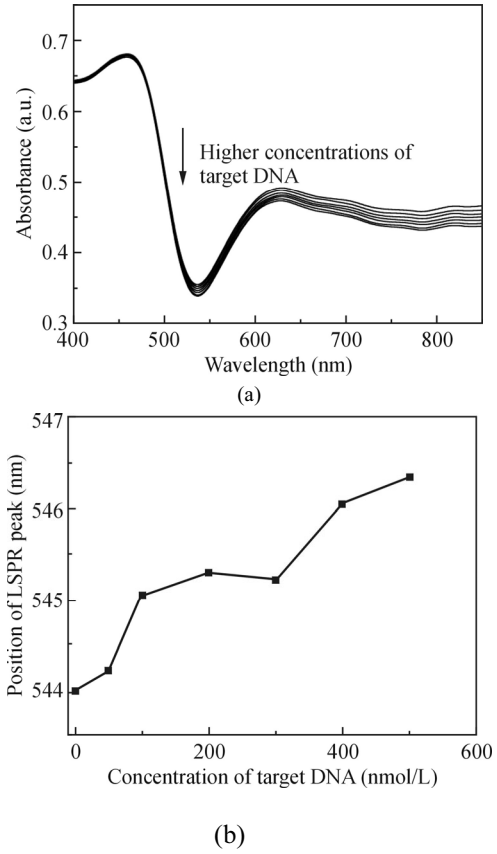



**Fig.5 (a) The absorbance spectra of $AuNS_{80\ nm}$ coated sensor for DNA concentration ranging from 0 to 500 nmol/L; (b) The relationship between position of LSPR peak and target DNA concentration**

higher RI sensitivity than the $AuNS_{20\ nm}$-modified one. The wavelength interrogation mode is used to characterize the sensitivity of sensor. The DNA hybridizition at the surface of AuNSs can be monitored successfully by the $AuNS_{80\ nm}$ coated fiber sensor. This study demonstrates the feasibility of DNA sensing by this LSPR fiber sensor. This sensor has the characteristics such as smaller sample consumption, simpler structure and easier fabrication compared with other optical fiber LSPR sensors. In addition, this sensor can be integrated with microfluidic system and has the potential for medical diagnosis.

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