The Design of a Nanometer Biosensor and Its Microfluidic Integration*

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Abstract*—A new biosensor structure, characterized by two layers of metal nano-particle, is proposed and fabricated with NSL (Nano-sphere Lithography) process. It improves sensitivity by exciting LSPR (Localized Surface Plasmonic Resonance) interactions between electromagnetic fields in the two layers. A simple microfluidic integration scheme for the sensor is designed for real-time and in-situ detection. As an important part of the scheme, a new microfluidic pumping mechanism is analyzed and verified with a prototype.

***Keywords*—biosensor, LSPR (Localized Surface Plasmonic Resonance), micropump, microfluidics**

I. INTRODUCTION

Bio-detecting technology plays important role in clinic diagnosing, medical robots, drug developing, pollution monitoring and many other applications. High sensitivity and good efficiency are two main requests. Traditional methods usually employ marking materials, such as radioactive isotope and fluorescent dyes, to indicate bio-molecules. This method suffers from inadequate reliability, and low efficiency.

The nano-structure based on LSPR (Localized Surface Plasmonic Resonance) principle is a new biosensor and became a good choice [1-8]. Such a sensor shows high sensitivity. With its characteristic of mark-free, the sensor gets rid of the dependence on the inter-reaction between objective molecules and mark materials that leads to inadequate reliability and complicated operation.

Furthermore, its structural agility makes it possible to improve performance easily. Up to now, the optimized structures of LSPR sensor are still under searching and researching. More newly born fabricating techniques will make much more possible structural arrangement, better performance and more function. In this paper, a new structure of LSPR biosensor attained by simply changing the original NSL (Nano-sphere Lithography) process [6] is discussed, since it shows potential of improving sensitivity.

By integrating LSPR biosensor into a microfluidic circuit, the real-time and in-situ detection ability could be exploited [9-10]. They are very important for many applications, accompanied with high efficiency and large throughput. Until now, the microfluidic control for LSPR biosensor is not full developed yet. In this paper, based on a simple microfluidic integration scheme with a LSPR biosensor, a suitable microfluidic actuation mechanism for this purpose is studied.

II. THE NANOMETER BIOSENSOR

A. Principle of the LSPR Biosensor

The bio-sensing principle of the sensor is based on LSPR phenomenon occurring in a metal nano-structure. On the surface of metal nano-particles in an electromagnetic field, there is an electron density wave that acts as a polarized vibrating vector. When the vector along the particle surface of incident light matches the inherent vector, LSPR occurs. It causes distinct optical characteristics different from traditional material. The energy of the incident light will be absorbed into the metal, resulting in a obvious drop of the transmitting energy that correspond to a valley in the transmission spectrum curve or a peak in the extinction spectrum curve. When the bio-molecules are bonded onto the metal surface, the frequency of LSPR will be changed, making the principle of using such a structure as biosensor feasible [5-6]. The commonly explanation now to the phenomenon is that the molecules changes the refractive index, in turn alters the vector of the incident light on the metal surface.

B. Design & Fabrication of the LSPR Biosensor

Haes A J and Duyue R P V realized metal nano-array, consisting of nano-particles on quartz, with NSL (Nano-sphere Lithography) fabrication process, and attained its extinction

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spectrum [6]. NSL shows great advantages of simplicity and low-cost.

Our attention is put on building more complicate structures by similar fabrication method. The key idea is that forming multi-layer nano-particle arrays may create interactions between different localized surface plasmonic fields.

The new metal nano-structure of biosensor fabricated with NSL method shown in Fig.1. The geometry detail and dimension attained by analyzing the SEM and AFM data are depicted in Fig.2. The structure consists of two metal layers, one is on quartz surface, and the other is the metal array on nano-sphere surface.

Another advantage of the structure in Fig.2 is the large metal area that will remarkably increase the bio-bonding probability. This could makes detecting sample of very low concentration possible.

By choosing diameter of spheres and depositing time, the dimension of the structure could be adjusted. The property of metal surface is modified by subsequent chemical treating process.

C. *Test Result of the Biosensor*

Since the biosensor principle is based on the change of refraction index on the metal surface, we coat a layer of inorganic colloid of about 7nm on the array. This method is simple but only suitable for verifying the shift of LSPR resonance curve. The colloid consists of silicon dioxide and titanium dioxide. Their proportion can be adjusted to make different refraction index. Fig.3 shows the transmission spectrum.

By contrasting Fig.2 and the structure in reference [6] that is in same dimension, it is found that the transmission spectrum curves have all two peaks. But the existence of the metal layer on nano-spheres makes the left peak and valley narrow and prominent. In other words, the multi-layer structure in Fig.2 shows higher sensitivity in left peak and valley.

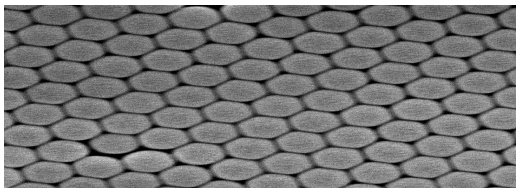

Figure 1. The SEM image of Ag@ polystyrene &quartz

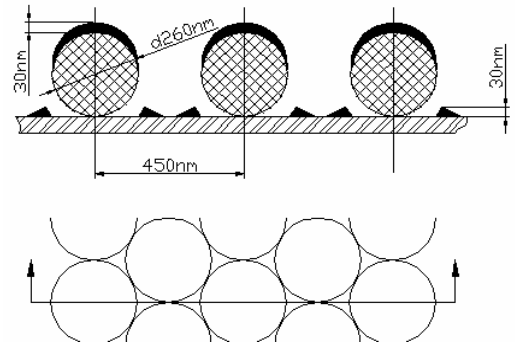

Figure 2. The geometry detail of the array of Ag@ polystyrene &quartz

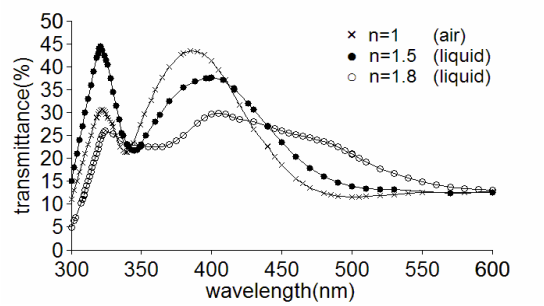

Figure 3. The transmittance spectrum for multi-layer array

In Fig.3, the wavelengths of left peaks are 323.13nm, 322.93nm and 332.20 nm, corresponding respectively to covering layer of air, colloid layer of refraction index 1.5 and that of refraction index 1.8. Its maximal shift is 9.07nm. The wavelength data of right peak are 388.08nm, 399.00nm and 406.76 nm respectively.

We estimated the sensitivity of peak with parameter *K* that equals to the maximal wavelength shift divided by half width of the peak (or valley) when refraction index is 1. By calculation, *K* is 0.236, 0.77 and 0.93 respectively for right peak, left peak and valley. The valley corresponds to the peak in extinction spectrum. It is obviously that the sensitivity near LSPR frequency is highest.

III. MICROFLUIDIC INTEGRATION SCHEME & PUMPING MECHANISM

A. *Microfluidic Integration Scheme*

In order to develop real-time and in-situ detection, LSPR biosensor has to be mounted in a microfluidic biochip. Up to now, the microfluidic control scheme for LSPR sensor is still not fully developed yet.

Fig.4 shows a simple scheme for application of LSPR biosensor. The fluid flows along micro channel, passing over the sensor and discharging out of chip finally. The light is lead to the nano-particles with fiber, gotten by a spectrum meter. The data were then sent to the computer for analyzing.

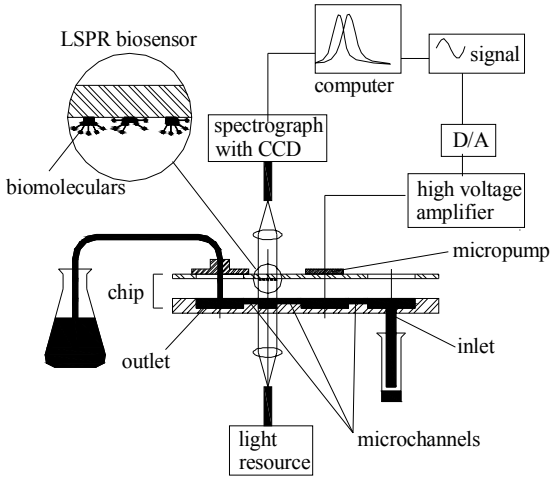

Figure 4. The microfluidic integration scheme of the bio-sensor

Since a layer molecules acting as "acceptor" has been previously bonded on the metal surface by chemical processing, the objective bio-molecules in the fluid will be captured when passing over the metal nano-particles.

The absorbing, reacting and removing of the molecules could be instantly observed. This is important for discovering interplaying detail among different molecules and cells.

A key problem is that how to ensure the accuracy, repeatability and reliability. Since only part of the object molecules can be captured, how to control the exact proportion of the molecules being bonded to the metal surface? In other words, how can the concentration been exactly detected?

Discrimination to different component in fluid, deposition along the micro channels and the turbulence on the sensor are important influencing factors. Designing proper micro-channel structure to form steady laminar flow on the sensor is a reasonable choice and an easy way for avoiding turbulence.

B. Pumping Mechanism

Two most common pump for microfluidic application nowadays, electroosmotic pump and mechanical pump with valves, may be not suitable for real-time or in-situ bio-detection. Electroosmotic pump shows discrimination to different component in sample [11]. The intermittent flow of objective molecules and unsteady concentration of sample affect the response time and detection accuracy. The valve movement in a mechanical pump may destroy big molecules, and cause obvious flow fluctuation [12-13].

A good choice is micro mechanical valveless pump, although its output pressure is not very high yet. It is acceptable because of no discrimination, no breakage to any molecules, and very low flow fluctuation [14-15].

In order to attain higher output pressure and to depress flow fluctuation further, we proposed a new structure of micro mechanical valveless pump. It characterized by a round-path chamber, and works on the establishing and strengthening of momentum predominance in one circular direction.

Fig.5 and Fig. 6 show the hydrokinetic simulation result. Vector marks in Fig.5 is much denser than Fig.6 in order to emphasize the clockwise momentum predominance in the chamber expanding process.

Such momentum predominance reduces the proportion of back flow in the chamber. It also increases the difference between resistances in two directions. On these effects, the output pressure would be increased, and the output fluctuation could be depressed.

The simulation results suggest also that the momentum predominance will be strengthened after every cycle (Fig.7 and Fig.8). So, the output pressure and the flow steadiness will be improved continuously. T is the period of a chamber expanding-contracting cycle.

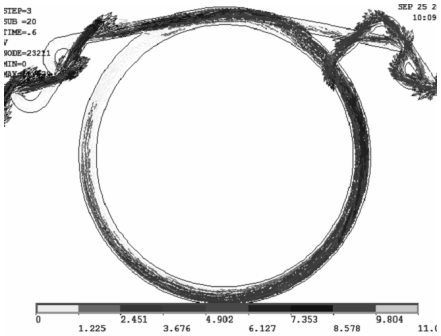

Figure 5. Clockwise momentum predominance in the expanding process of chamber (denser vector marks is used)

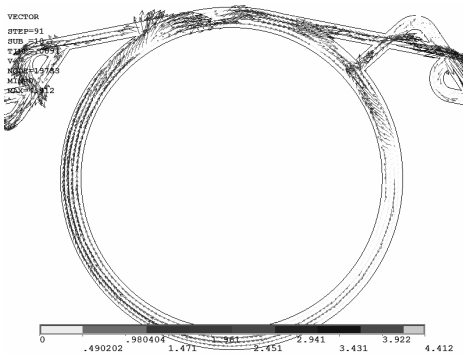

Figure 6. Keeping of the predominance in the contracting process of chamber

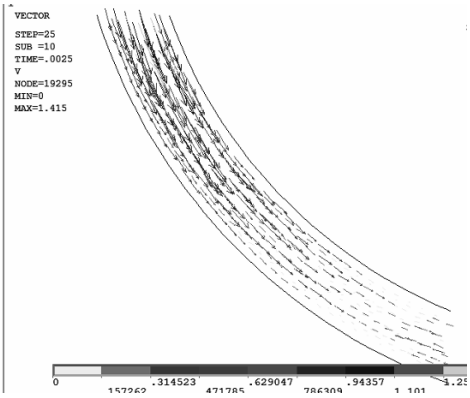

Figure 7. 0.25T moment of 1st cycle

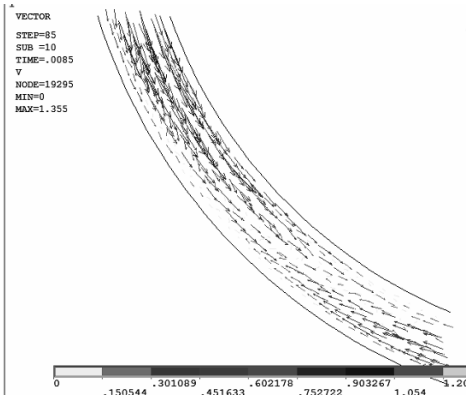

Figure 8. 0.25T moment of 4th cycle

C. Test Result of the pump

Such a pump made on a silicon substrate (Fig.9) shows maximal flow of 35×10^{-6}L/min and maximal output pressure of 1.52 kPa that is higher than that of the pump with a traditional column chamber (about 0.8kPa). The water level in outlet tube seems extremely stable under microscope.

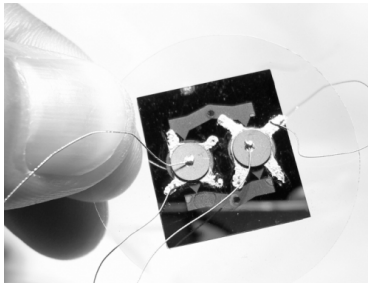

Figure 9. One of the micro-pump prototypes

IV. DISCUSSION

A new structure of LSPR biosensor is proposed, fabricated and tested. It is based on the key idea of creating interactions of different LSPR fields between two metal layers. The test data is analyzed. As a result, it shows the prospect of higher sensitivity than monolayer structure.

With different shape and dimension of metal nano-particles, different layer arrangement and layer number, LSPR results could be different. So, searching the best design is an important goal attracting us to study further.

By combing the sensor and microfluidic circuit, a simple integration scheme is proposed and its key problems are discussed. Forming steady laminar flow is considered to be very important for making the bio-detection exactly comparable and reliable.

As one of important part, the microfluidic pumping mechanism is especially studied. A micro mechanical valveless pump characterized by a round-path chamber is proposed and analyzed. Momentum predominance in one circular direction in the chamber will lead to higher output pressure and lower output fluctuation. The prototype is fabricated and tested.

However, up to present, there are still many problems in microfluidic integration of LSPR biosensor. Design should be improved and a lot of experiment should be carried out.

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