The Detection of Acetylcholinesterase Based on All-Dielectric Nanoantennas

Ao Ouyang\textsuperscript{1}, Huan Zhou\textsuperscript{1}, Pintu Ghosh\textsuperscript{1} and Qiang Li\textsuperscript{1,\ast}

\textsuperscript{1}State Key Laboratory of Modern Optical Instrumentation, College of Optical Science and Engineering, Zhejiang University, Hangzhou 310027, China
\textsuperscript{\ast}Corresponding Author Email: qiangli@zju.edu.cn

Abstract. High sensitivity detection of acetylcholinesterase (Ache) concentration is an important method for water pollution control and treatment. Compared with commonly used detection methods, the dielectric nanoantenna detection method is more direct, efficient, and safer. In this paper, we first studied a silicon disk dielectric antenna for the detection of Ache. We made the structure by electron beam lithography technology and set up the optical system of spectrum measurement. We used the electrical resonance position of its transmission spectrum to calibrate the refractive index of the surface environment, and the sensitivity reaches 222nm/RIU. We introduced the chemical bond coupling method to monitor the Ache concentration changing process, and there is no need to mark the detection substance by modifying the surface of the antenna structure and fixing the antibodies of the substance to be monitored. We achieved the specific monitoring process of Ache concentration, and the minimum detectable concentration is 0.01mg/ml.

1. Introduction

Acetylcholinesterase (Ache) is ubiquitous in the nervous system and plays an important role in signal transmission between neurons. In the treatment of Alzheimer's disease, the balance of acetylcholine in the patient's nervous system is usually regulated by the reversible inhibitor of acetylcholinesterase [1]. On the other hand, in the field of agricultural pollution control, phosphates in organophosphorus pesticides can inhibit the activity of Ache. Many biosensors based on Ache are used in the detection of organophosphorus residues in agriculture and food [2]. It is therefore necessary to develop a rapid and reliable method for Ache detection. At present, the most commonly used method in the detection of Ache is colorimetric detection [3], but the sensitivity of this method is low and more reactants are needed, there are also some other methods like the chemiluminescence method [4], fluorescence analysis [5], quantum dot method [6], electrochemical method [7], grating structure detection method [8], etc. However, these methods need complex operations and some even need chemical labels which may damage analyses, so it is still challenging to develop a simple, rapid, and sensitive method for Ache analysis.

Recently, the optical biosensor based on the surface plasmon resonance (SPR) has much application in detection and this method has many advantages in protein detection, such as protein-carbohydrate [9], protein antibody [10], and DNA [11]. Compared with the biosensor based on SPR, all-dielectric nanoantenna can show great natural advantages due to its material characteristics [12], this method can avoid thermal effects, which have greater potential in protein detection. In this paper, we develop a high sensitivity, reliable and quick method for detecting Ache concentration based on all-dielectric nanoantenna. To show the specificity in detection, the detection of Ache and BSA are compared.
2. Methods

2.1. Materials and Sample Preparation

Purity 98% concentrated H\textsubscript{2}SO\textsubscript{4}, purity 30% hydrogen peroxide solution, 99% purity disodium hydrogen phosphate dodecahydrate, anhydrous sodium dihydrogen phosphate, acetone, anhydrous ethanol, purity 97% succinic anhydride, 98% purity 3-aminopropyltriethoxysilane (APTES), 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (EDC), N-hydroxy succinimide (NHS), polyclonal rabbit Ache antibody, acetylcholinesterase protein solution (using PBS buffer). Experimentally, we fabricate silicon nano-disk arrays using electron beam lithography (EBL) and inductively coupled plasma reactive ion etching (ICP-RIE) on a backside polished silicon substrate. Figure 1(a) shows the scanning electron microscopy (SEM) image of the top view of a section of a typically fabricated array.

2.2. Instrumentation

Antibody incubator, vortex mixer, the spectrum is measured by a self-built optical system (Wavelength range: 1450 nm-1500 nm).

2.3. Surface Functionalization

More specifically, this biosensing process involves three steps, namely surface activation, linker functionalization, and antibody capture, which are presented in Fig 1(b).

Hydrophilic treatment part: we mix the concentrated sulfuric acid and hydrogen peroxide in a volume ratio of 3:1 and place the sensor chip in the mixture at room temperature for more than 2 hours. Then we clean the sample surface thoroughly with ionic water and dry it with high-purity nitrogen.

APTES treatment part: we immerse the sensor chip in the acetone solution containing 2% APTES for more than 3 hours and wash it with acetone, anhydrous ethanol, and deionized water successively. Then we blow the sample dry with high purity nitrogen.

Carboxylation treatment part: we soak the sensor chip treated by APTES in saturated succinic anhydride solution and wash them with anhydrous ethanol and deionized water two times respectively.

EDC / NHS activated carboxyl part [13]: we mix EDC (75mM) and NHS (25mM) in a volume ratio of 3:1 and immerse the sensor chip in it for 20 min. Then we add 30 µl of antibody drops to the surface of the sensor chip and place the sensor chip in the antibody incubator. We drop a few drops of water in the incubator to keep the humid environment in the incubator and put the sample in a 37°C water bath for 30 min.

![Figure 1](image-url)

Figure 1. Scanning electron microscopy images of (a) the top view of a section of a typical sample of silicon nano-disk arrays used in the experiment. (b) Steps of Surface Functionalization: step 1: surface activation by creating hydroxyl radicals (-OH) on the surface of the sample using the concentrated sulfuric acid and hydrogen peroxide, step 2: amination using APTES, step 3: carboxylation using saturated succinic anhydride solution.
2.4. Numerical Simulation

Figure 2(a) shows the calculated spectrum using FDTD and the illustration shows the electric field diagram and the magnetic field diagram respectively. We can see it is a magnetic resonance at 1482 nm. We model our sample as an infinite array of crystalline silicon nano-disks (using FDTD) with h = 220nm, d = 730nm, and lattice periodicity a = 960nm. The silicon nano-disks are on a SiO$_2$ substrate with a thickness of 1 µm and an infinitely thick silicon layer. Figure 2(b) shows the resonance peak varies with the refractive index of the environment. As is shown in Fig 2(b), when the refractive index changes from 1.33 to 1.4, the magnetic resonance position moves from 1571nm to 1594nm and the sensitivity reaches 328nm/RIU.

![Figure 2](image)

Figure 2. (a) The calculated spectrum shows the transmission of an infinite array of crystalline silicon nano-disks and the illustration shows the electric field diagram and the magnetic field diagram. (b) The resonance peak varies with the refractive index of the environment.

3. Results

After the antibody is incubated, the sample modified with Ache antibody is rinsed with PBS buffer for 2 min and then we add 0.01mg/ml, 0.05mg/ml, 0.1mg/ml Ache solution to the sample in turn with each drop of 30µl. Then we put it in the antibody incubator and store it in a water bath at 37°C for 30min. Before measurement, the sample should be washed with PBS buffer for 1 min and dried with nitrogen. Figure 3(a) shows the change of the transmission spectrum with the change of Ache antigen concentration. When the concentration of Ache increases, magnetic resonance peak redshift. After the modification of the antibody, the magnetic resonance position is at 1469nm and the redshift corresponding to 0.01mg/ml Ache is the largest. After the antigen test, washing the sample with PBS buffer, the resonance peak is still at 1482nm, which proves that the antibody-antigen has good binding stability. With the help of a silicon dielectric antenna, we can dynamically observe the process of adding Ache concentration in practical operation. The minimum detectable concentration is 0.01mg/ml and the corresponding redshift is 4nm.

To further verify the modification of the Ache antibody on the surface of the sample, bovine serum protein (BSA) is used to compare the specificity of the antibody, using the same method of surface modification. After the antibody is incubated, the sample modified with Ache antibody is taken out from the antibody incubator and rinsed with PBS buffer for 2min. Then we add 30µl 0.1mg/ml BSA solution to the sample. Before measurement, the sample should be washed with PBS buffer for 1 min and dried with nitrogen. Figure 3(b) shows the contrast transmission spectra of Ache and BSA. When the BSA solution is dripped, the resonance peak does not move. When Ache solution with a concentration of 0.1 mg/ml is dripped, the resonance peak appears obvious redshift, which shows that the detection scheme has specificity.
Figure 3. (a) The change of transmission spectrum with the change of Ache antigen concentration, before antibody modification (blue), after antibody modification (orange), 0.01mg/ml Ache (yellow), 0.05mg/ml Ache (purple), 0.01mg/ml Ache (light blue), washing with PBS buffer (green). (b) The contrast transmission spectra of Ache and BSA, before antibody modification (blue), after antibody modification (orange), 0.01mg/ml BSA (yellow), 0.01mg/ml Ache (purple).

4. Conclusions
In conclusion, we have demonstrated the quantitative and qualitative detection of Ache based on magnetic resonance of the silicon meta-surface. Transmission characteristics of a three-layer dielectric antenna with silicon disk layer-silicon oxide layer-silicon substrate layer are analyzed by FDTD. The disk antenna structure has a very obvious magnetic resonance position and the sensitivity of the silicon disk dielectric antenna structure to the surface refractive index can reach 222nm/RIU. We use this structure to detect Ache, and the minimum detectable concentration is 0.01mg/ml. This silicon platform has many advantages, such as low conductivity, high resonance quality, and bio-compatibility, so all-dielectric nanoantennas can lead to applications in protein detection, more sensitive molecule detection, etc.

5. Acknowledgments
This work was supported by the National Key Research and Development Program of China (2017YFE0100200), the National Natural Science Foundation of China (Grant No.61950410608) and the Fundamental Research Funds for the Central Universities. The authors thank Prof. Pavel A Belov from ITMO University, Russia and Prof. Ravindra Kumar Sinha from CSIR-Central Scientific Instruments Organization, India for fruitful discussions.

6. References
[1] Bartolini M, Bertucci C, Cavrini V and Andrisano V 2003 J. Biochem Pharmacol. 65(3): 407-416.
[2] Liu Y, Wang C, Gui W, Bi J, Jin M and Zhu G 2009 J. Ecotox Environ Safe. 72(6): 1673-1679.
[3] Magnotti R A, Eberly J P, Quarm D E A and McConnel R S 1987 J. Clin Chem. 33(10): 1731-1735.
[4] Liu Q, Peng Y, Xu J and Ma C 2017 J. Chemselectrochem. 4(7): 1768-1774.
[5] Liao D, Wang Y, Chen J, Zhou H Li Y and Yu C 2013 J. Anal Chem. 85(5): 2667-2672.
[6] Zhan Y, Yang J, Guo L, Luo F, Qiu B, Hong G and Lin Z 2019 J. Sens Actuator B-Chem. 279: 61-68.
[7] Wang H, Wang J, Timchalk C and Lin Y 2008 J. Anal Chem. 80(22): 8477-8484.
[8] Tian T and et al 2014 J. ACS Appl Mater Interfaces. 6(17): 15456-15465.
[9] Smith E A, Thomas W D, Kiessling L L and Corn M 2003 J. J Am Chem Soc. 125(20): 6140-6148.
[10] Kyo M, Usuiaoki K and Koga H 2005 J. Anal. Chem. 77(22): 7115-7121.
[11] Shumakerparry J S, Zareie M H, Aebersold R and Campbell C T 2004 J. Anal Chem. 76(4): 918-929.
[12] Bontempi N and et al 2017 J. Nanoscale. 9(15): 4972-4980.
[13] Jang L and Keng H 2008 J. Biomed Microdevices. 10(2): 203-211.