Research on optical biosensor with up-converting phosphor marker

Yongkai Zhao (赵永凯)[1,4], Lei Zhou (周 蕾)[2], Jing Wang (王 静)[3], Lihua Huang (黄立华)[1], Zhongqiang Yan (闫中强)[2], Huijie Huang (黄惠杰)[1], Ruifu Yang (杨瑞馥)[2], Lei Liu (刘 蕾)[1], Bingqiang Ren (任冰强)[1], and Xiangzhao Wang (王向朝)[1]

[1]*Shanghai Institute of Optics and Fine Mechanics, Chinese Academy of Sciences, Shanghai 201800*

[2]*Institute of Microbiology and Epidemiology, Academy of Military Medical Sciences, Beijing 100071*

[3]*Chinese Academy of Inspection and Quarantine, Beijing 100025*

[4]*Graduate School of the Chinese Academy of Sciences, Beijing 100039*

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An optical biosensor with up-converting phosphor (UCP) marker is developed for the sensitive rapid immunoassay to the specific biomolecule. UCP can emit visible light when excited by infrared light. Through detecting and analyzing the content of UCP particles on the test strip after immunoreaction, the concentration of target analyte in the sample can be obtained. The detection sensitivity to plague IgG is better than 5 ng/ml; to plague FI-Ab is better than 100 pg/ml; to plague *Yersinia pestis* cell is better than 3×10^4 CFU/ml. Good linear response characteristics and an excellent correlation ($R^2 \geq 0.95$) have been verified by quantitative detection results. In the practical application, detection results to 167 analytic samples have an excellent consistency with those obtained by reverse hemagglutination test. The up-converting phosphor technology (UPT) based biosensor has stable, reliable, and sensitive performances. It can meet the need of various bioassay applications.

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Optical biosensors, which use light to detect the effects of chemicals on biological systems and feature high sensitivity and specificity, are important tools in biotechnology, immunological diagnostics, anti-bioterrorism, and environmental monitoring[1−4]. There are two types of optical biosensors, marked and non-marked, depending on whether or not they use an optical marker.

There now exist a variety of optical biosensors for the detection of a target analyte with different markers, such as radioisotopes, fluorescent dyes, enzymes, and colloidal gold. However, these markers have drawbacks in one way or another such as decay, unsafety, autofluorescence, photobleaching effect, and un-quantifiability[5].

Up-converting phosphor (UCP) particles are a novel kind of biological marker[6]. They are submicron crystal particles by doping the rare-earth element into the crystal lattice. UCP can emit visible light wavelength when excited by infrared light, i.e., up-conversion, whose process is phosphorescence emission after two-photon or multi-photon absorption. It is very easy to separate UCP's phosphorescence emission from exciting light between which there is a large anti-Stokes shift. An optical biosensor which uses UCP particles as the marker is regarded as up-converting phosphor technology based biosensor (UPT-based biosensor). For UCP's advantages such as sensitivity, flexibility, stability, safety, and quantifiability, the UCP technology based biosensor can overcome the limitations of other biosensors where conventional markers are used.

In this paper, an UPT-based biosensor has been developed, where the UCP material is Yb^{3+},Er^{3+}:$NaYF_4$. The $NaYF_4$ host lattice has been widely recognized as one of the most efficient up-converting lattices specifically when it is doped with Er^{3+} and Yb^{3+}[7]. The absorption peak wavelengths of this material is 980 nm, and there are two emission peaks at the wavelengths of 541.5 and 670 nm. The spherical UCP particle with homogeneous diameters within 200—300 nm has been synthesized, which can meet the requirement of the immune chromatography and optical quantitative detection. After a series of surface modification and functionalization, these UCP particles can be attached to various ligand biomolecules, such as antigen, antibody, etc.[8].

The measured object of the biosensor is the UPT immune chromatographic test strip, which is the reactor carrier for the biological active molecule marked with UCP particles to proceeding immunoreaction. A test line and a control line are included in the scanning window on the test strip, as shown in Fig. 1(a). Using the infrared laser as the exciting light, the UPT-based biosensor can detect the stimulated luminescence (phosphorescence) intensity of UCP particles combined on the area around test line and control line. Then the concentration of the target analyte in the sample can be calculated.

The content of UCP particles combined on the test line may not correspond to the concentration of target directly for many reasons[9]. Because the reaction

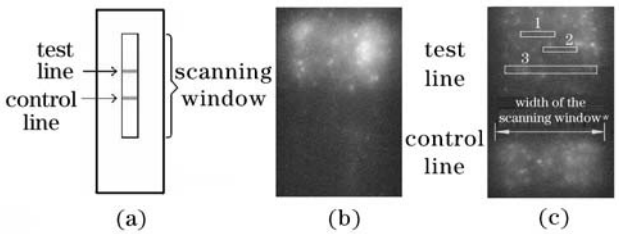

Fig. 1. Stimulated luminescence images of UCP particles on different test strips.

condition of test line and control line on the same test strip is same, the ratio of test line signal T to control line signal C is used as the judgment information. Through detecting a series of standard samples with known concentration, a linear equation between T/C and the concentration of target analyte could be fit as the calibration curve of this biosensor.

The stimulated luminescence images of UCP particles on different test strips' surfaces, illuminated with uniform laser beam, were obtained by a sensitive black-and-white CCD camera, as shown in Figs. 1(b) and (c). The longer luminescence areas in the upper of two images are test lines, while the shorter in the lower are control lines. In Fig. 1(b), the luminescence of the test line is bright, but the control line is very dim, which shows that UCP particles are almost combined on the test line. It is an instance that the concentration of the target analyte in the sample is very high. In Fig. 1(c), there are UCP particles on both lines, indicating the concentration is lower than that of Fig. 1(b).

In order to obtain better working performances, the optical system of the UPT-based biosensor is different from the prototype reported earlier[9]. 1) A 980-nm laser diode with a power of 80 mW was used, which is higher than that of the prototype. 2) An adjustable lens was used in the exciting optics to adjust the area of the focal line, hence the focal area is controlled nearly to 2.8 mm $\times$30 μm, as the focal line 3 highlighted in Fig. 1(c), which is bigger than the prototype's. This modification is applied to eliminate the influence of the non-uniform distribution of the UCP particles along the direction of the width of the scanning window. 3) The focusing/collimating lens with larger numerical aperture (NA) was designed to raise the collecting efficiency to the phosphorescence. 4) The optical system can availably collect both phosphorescence of the Yb^{3+},Er^{3+}:$NaYF_4$ at the wavelengths of 541.5 and 670 nm. 5) The confocal field stop before the photodetector was a slit of 3 mm$\times$60 μm, which is a little bigger than the theoretical value based on the comparison test results.

The time-dependent luminescence curve of UCP particles is given in Fig. 2, where the exciting laser is modulated to a square wave with a frequency of 50 Hz. According to the figure, the stimulated luminescence curve following the exciting light includes a rise period, a stable period, and a fall period. The rise time is about 2.5 ms, and the fall 3 ms. Figure 3 shows the curve of the phosphorescence intensity versus the exciting light intensity, which exhibits a good linearity. No photobleaching

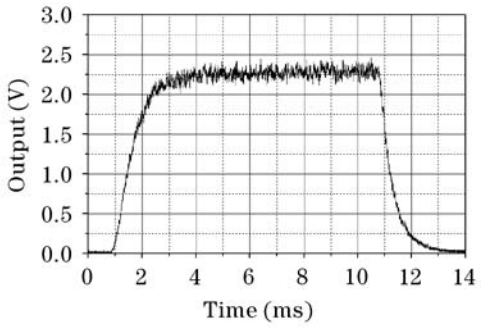

Fig. 2. Stimulated luminescence curve of UCP particles under exciting light with 50-Hz modulation frequency.

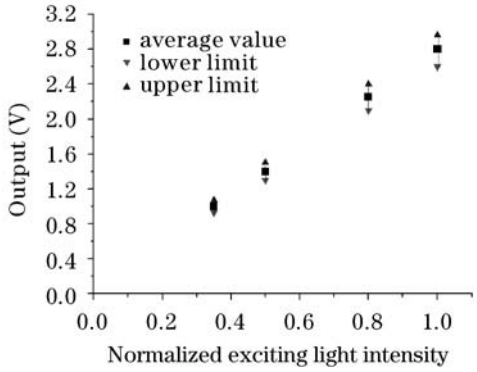

Fig. 3. Average output values and variance ranges in stable period of same UCP particles under exciting light with different intensities.

effect was observed in our experiment.

The relative variance of the signal during the stable period is about $\pm$7% relative to the average value. This variance is adverse to the linear detection range and sensitivity of the biosensor. If the concentration of the analyte is near the minimum detection limit, the variance of signal could affect the T/C value, which causes a false result. To obtain real, stable stimulated luminescence of UCP particles, the control system of the biosensor was set to sample the signal 2.5 ms after the laser was turned on. On the other hand, to minish the effect of variance, multipoint continuous sampling on each point to the stimulated luminescence during the stable period was carried out in the software of the biosensor, and then the average was calculated as the final signal value.

The performance of the UPT-based biosensor was tested. To test its stability, one test strip was repeatedly scanned 11 times. The results are shown in Fig. 4. The variation coefficients of T and C are less than 1.5%, and for T/C it is less than 1%. The result shows that the biosensor has excellent repeatability, which is much better than those of the prototype.

Figure 5 shows the quantitative detection results of two analytes. The expressions of linear fitting by least-squares method and correlation coefficients (R^2) are shown in the figure. Figure 5(a) is the result of the plague IgG in a sandwich immunoreaction format, which shows the sensitivity of the biosensor is better than 5 ng/ml. The linear response relation is obtained in the concentration range of 5—50 ng/ml. Figure 5(b) is the result of

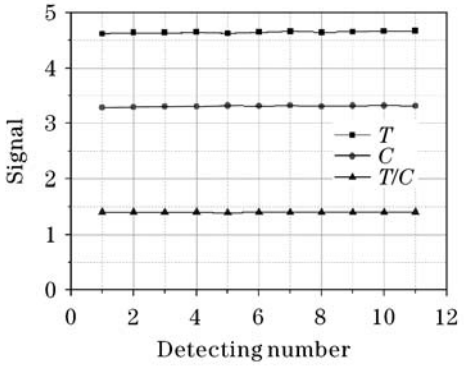

Fig. 4. Repeatability test results of the biosensor.

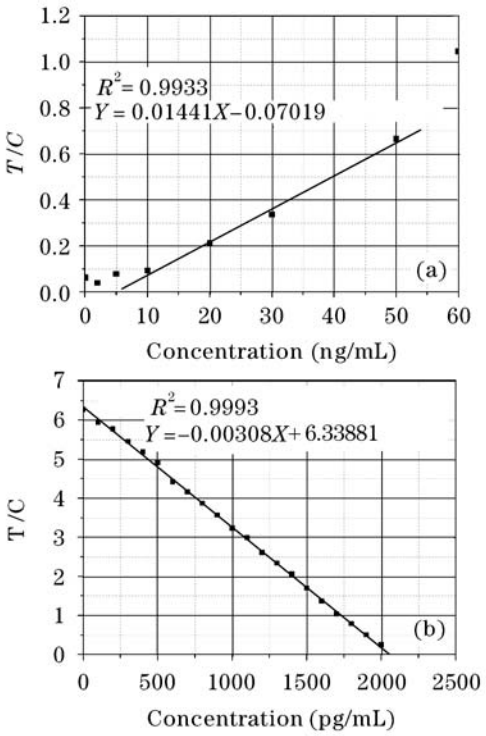

Fig. 5. Quantitative detection results of the biosensor for detecting the plagues IgG (a) and FI-Ag (b).

the plague FI-Ag in a competitive mode, which shows that the sensitivity is better than 100 pg/ml. According to Fig. 5, the sensitivity of the biosensor reaches ng/ml even pg/ml level; good linear relationship in the tested concentration range, and excellent correlation ($R^2 \geq 0.95$) are realized.

The whole cell detection (WCD) is the most rapid, direct method in the field of epidemic sources. To evaluate the practical application ability of the biosensor, the quantitive detection to the plague *Yersinia pestis* EV76 cell was performed. The result shown in Fig. 6 indicates that the sensitivity of the biosensor to plague *Yersinia pestis* cell reaches 3×10^4 CFU/ml (CFU: colony-forming unit); the good linear response characteristic ($R^2 > 0.97$) has been obtained in the concentration range of $10^4 - 10^6$ CFU/ml.

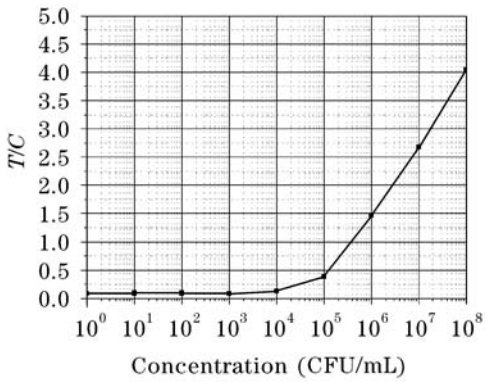

Fig. 6. Practical quantitative detection results of the biosensor for detecting the plague *Yersinia pestis*.

167 analytic samples from various animals' visceras were detected for the plague *Yersinia pestis* cell by the biosensor, and T/C value was used as the final result of quantitative detection. $T/C = 0.5$ is set as the threshold (cutoff value) for judgment. A sample whose T/C value is higher than 0.5 is positive, otherwise negative. 20 samples are positive according to threshold. The detection result has an excellent consistency with that by reverse hemagglutination test, the coincidence coefficient is nearly 100%.

In conclusion, the UPT-based biosensor has high detection sensitivity to different analytes, as well as stable and reliable performances. Its good linear response characteristics and excellent correlations have been obtained by quantitative detection results. In the practical WCD application, detection results have an excellent consistency with those obtained by reverse hemagglutination test. The results above show that this UPT-based biosensor can meet the need of some various applications, such as rapid immunoassay, chemical and biological detection, and so on.

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