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Microcantilever array instrument based on optical fiber and performance analysis

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We developed a microcantilever array biosensor instrument based on optical readout from a microcantilever array in fluid environment. The microcantilever signals were read out sequentially by laser beams emitted from eight optical fibers. The optical fibers were coupled to lasers, while the other ends of the fibers were embedded in eight V-grooves with 250 μm pitch microfabricated from a Si wafer. Aspherical lens was used to keep the distance between lasers. A programmable logic controller was used to make the system work stably. To make sure that the output of lasers was stable, a temperature controller was set up for each laser. When the deflection signal was collected, lasers used here were set to be on for at least 400 ms in each scanning cycle to get high signal-to-noise ratio deflection curves. A test was performed by changing the temperature of the liquid cell holding a microcantilever array to verify the consistent response of the instrument to the cantilever deflections. The stability and conformance of the instrument were demonstrated by quantitative detection of mercury ions in aqueous solution and comparison detection of clenbuterol by setting test and reference cantilevers. This microcantilever array detection instrument can be applied to highly sensitive detection of chemical and biological molecules in fluid environment. *Published by AIP Publishing.* [<http://dx.doi.org/10.1063/1.4994736>]

I. INTRODUCTION

With the rapid development of micro-nano manufacturing technology, the microcantilever sensor technology has been increasingly applied in the last few decades. This sensor technology has the advantage of label free, real time, *in situ*, and high sensitivity. Main techniques for the readout of the sensor response include optical lever method,¹ capacitance readout,² piezoelectric,^{3,4} piezoresistive,^{5,6} and optical waveguide cantilevers.^{7,8} The microcantilever sensor has been applied to the detection of DNA,^{9,10} proteins,^{11,12} small molecules^{13,14} and ions,^{15–17} activity analysis of microorganisms,¹⁸ and drug screening and monitoring.^{19–22} In recent years, the microcantilever sensor technology has been playing an increasingly important role in the research area of biology, chemistry, food safety, and environment monitoring.

The optical lever method is the most widely used technique and can be applied to both gas and liquid environment. Only tens to hundreds of microliters of sample is needed for the analysis procedure. This method can be used to detect the static deflection of the microcantilever. The deflection is caused by variation of the cantilever surface stresses, which is generated by intermolecular interactions between the absorbed molecules on the microcantilever surface. The deflection signal of a microcantilever also contains lots of noises, including temperature instability, surrounding vibration, and especially the uncertain reactions of the biochemical molecules on the microcantilever surface. These noises are extremely difficult to eliminate by noise signal processing. Adding reference

cantilevers by using microcantilever array is one of the simplest and most effective solutions. Nowadays, the microcantilever array system is applied to resolve the existing problems of the device with a single microcantilever. By comparing signals of reference cantilevers and test cantilevers, one can try to eliminate these noises. Meanwhile, using a microcantilever array containing more microcantilevers, which ensures detection carried out in the same environment, makes the repeated work more efficient and accurate, and one can detect different target molecules simultaneously by modifying corresponding antibodies on different cantilevers of one array chip.

Multiple light sources or a single moveable laser will be needed for detecting a microcantilever array. Vertical Cavity Surface Emitting Lasers (VCSELs) have been utilized as the array light sources,²³ they are quite compact and can be integrated as an array which is directly corresponding to the microcantilever array. But it is not convenient to control their temperature to stabilize the output. A movable laser was used as the light source of the microcantilever array by mounting the laser diode on voice coil actuators.²⁴ Two perpendicular linear voice coil actuators were used, which allowed us to detect the displacement of the whole cantilevers. The optical lever method is extremely sensitive, and the measurement accuracy reaches the nanometer level. The mechanical translational method has a risk of displacement errors which will cause noise, or even a false signal. A resonating reflector was used to scan the laser across the microcantilever array.²⁵ The reflector will change the incident angle of the laser and this will change the length of the laser beam reflecting from the cantilever slightly, causing difference in the deflections of the cantilevers. An aligned optical fiber array was used as a sequential light source of the microcantilever array.²⁶ But it was built

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with a large noise about $1\ \mu\text{m}$. The scanning cycle was not studied to obtain a stable and low-noise signal because it takes some time for the lasers to achieve a stable output after being lit.

In this work, a microcantilever array instrument based on optical readout was built by using eight optical fibers coupled to lasers as light sources, and temperature controllers were used to keep the temperature of the lasers and liquid cell stable. The signal noise caused by lasers working in different scanning cycles was analyzed to determine a reasonable scanning cycle. Then the cantilever deflections caused by temperature change were measured to verify that the instrument got a consistent response with each cantilever. Quantitative detection of mercury ions and immune detection of clenbuterol were carried out by the sensor instrument at last.

II. INSTRUMENT DESIGN

Figure 1 shows a schematic drawing of the instrument. Eight single mode diode lasers (2 mW power, 650 nm wavelength, HYST, China) were mounted on eight Peltier elements which were used for laser temperature stabilization. Each laser was coupled to a multimode optical fiber (core diameter $50\ \mu\text{m}$). The coating layer of the ends of the fibers was removed and the ends of the fibers were aligned and sealed in eight V-grooves with $250\ \mu\text{m}$ pitch manufactured from the Si wafer. Lenses were used to focus the light beams emitting from the fibers on each cantilever tip, respectively. In order to eliminate the spherical aberration, aspherical lens was used here. The position of the reflected beam was measured by using a quadrant photodiode detector, also called the position sensitive detector (PSD). The accuracy of the PSD used here is $0.1\ \mu\text{m}$, and the theoretical accuracy of the cantilever deflection detected by the optical lever method is $0.33\ \text{nm}$.

The central control unit of the instrument was a programmable logic controller (PLC, Siemens, s7-200). The PLC has been widely used in the industrial control field, and it has powerful and reliable data processing and control functions which could make the experiment procedure more stable. The PLC controlled the majority working processes of the instrument, including sequential control of the light sources, temperature control, and data acquisition, processing, and

storage: (1) The PLC provided a stable operating voltage for the lasers, adjusted the operating power, and set the working time of the lasers to make sure that the PSD collected signals with improved signal to noise ratio. (2) The PLC provided a closed-loop voltage for the Peltier elements to steady the operating temperature of the lasers and the temperature of the liquid cell which held the microcantilever array. (3) The PLC collected data at the set time accurately. CenturyStar configuration software (Chancla, China) was applied to control the sensor system. The deflection signals were acquired from the PSD, stored into a computer, and displayed on the screen in real time.

A liquid cell was developed which used a spring strip to hold the microcantilever array. The volume of the liquid cell was $200\ \mu\text{l}$. It will save reagents and make sure that the liquid can be exchanged at a gentle speed in the cell while the flow rate is $1\ \mu\text{l/s}$. A Peltier element was settled to keep the temperature of the liquid cell with an accuracy of $0.05\ ^\circ\text{C}$. It was under the bottom of the cell at a distance of $2\ \text{mm}$ away from the microcantilever array. The whole platform was placed indoors at a temperature of $25.0\ ^\circ\text{C}$ controlled by an air conditioner.

III. ANALYSIS AND IMPROVEMENT

A. Microcantilever array

The microcantilever array used here is the Si cantilever array with 8 cantilevers (Micromotive, Germany). The upper surface of the cantilevers is precoated with $2\ \text{nm}$ titanium and $20\ \text{nm}$ gold. Each cantilever is $500\ \mu\text{m}$ long, $90\ \mu\text{m}$ wide, and $1\ \mu\text{m}$ thick. The pitch of the cantilevers is $250\ \mu\text{m}$.

B. Analysis of noises from lasers

In consideration of reducing signal noise, it is essential to acquire the signal after the output of the laser is stable. It will take some time for the lasers to achieve a stable output in the sequential readout working mode here. Figure 2(a) shows how the lasers work in a scanning cycle. Each laser is on for the same time period ($T\ \text{ms}$) and then off for $20\ \text{ms}$ before the next laser is on. The deflection signal of each cantilever is acquired at just $10\ \text{ms}$ before the laser is off. In order to find out how long the output of the lasers would be stable after being lit, the

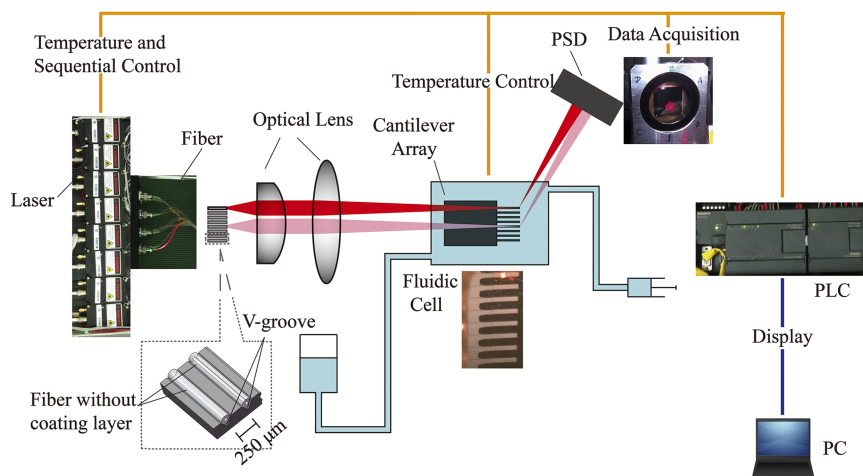


FIG. 1. Schematic of the detection system.

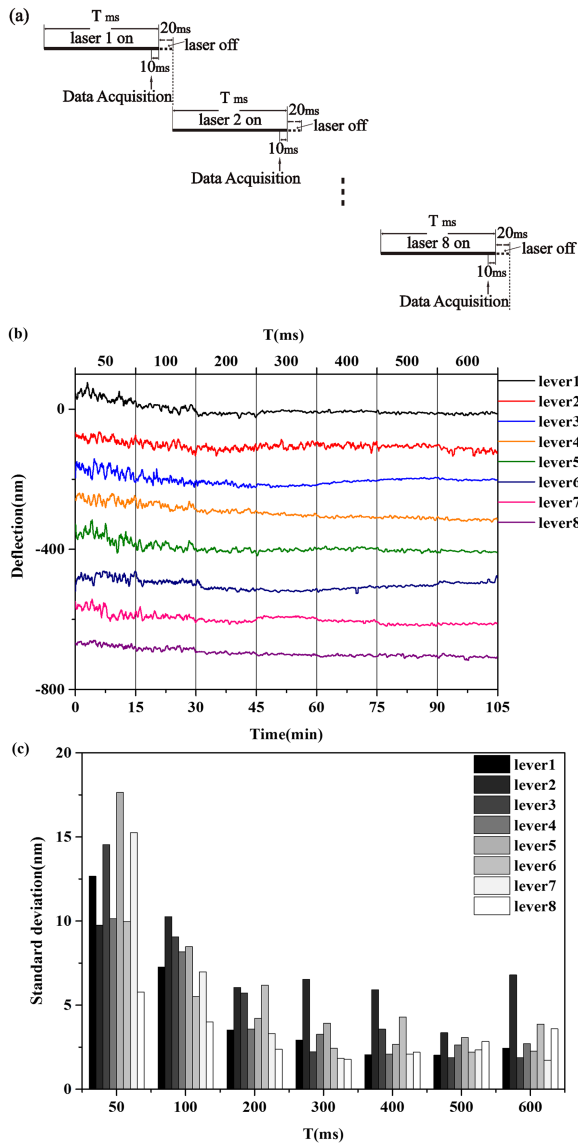


FIG. 2. Signal noise analysis of different scanning cycles. (a) Laser working process within one scanning cycle. Each laser is on for the same time period (T ms) and off for 20 ms before the next laser is on. The deflection data of each cantilever on a chip are acquired at 10 ms before the laser is off. (b) Deflection signals of the cantilevers with different “ T ” Time period “ T ” was changed every 15 min: 50, 100, 200 . . . until 600 ms. Bottom axis is the time axis while top axis shows the time period “ T .” For clarity, curves are separated vertically by an interval of 100 nm at 0 min and in order from lever1 to lever8. (c) Standard deviations of the deflection signals of different “ T ”

cantilever deflection signals were acquired while each laser was on for different “ T ”

A cleaned new microcantilever array was settled into the liquid cell filled with deionized water, and the temperature of the liquid cell was set at 25.00 ± 0.05 °C. Time period “ T ” was changed every 15 min: the duration time in each scanning cycle that every laser was on was 50, 100, 200 . . . until 600 ms. The deflection signal of each cantilever was acquired at 10 ms before the laser was off. The signal curves including noises with different “ T ” are shown in Fig. 2(b). The bottom axis is the time axis while the top axis shows the time period “ T ” at different times. The column diagram in Fig. 2(c) shows the standard deviations calculated from the deflection signals of different “ T .” The standard deviation stands for the noise

amplitude of the signal, which is also displayed by fluctuations in the signal curves in Fig. 2(b). As is shown in Fig. 2(c), the standard deviations decreased when “ T ” was changed from 50 to 400 ms and stabilized when “ T ” was greater than 400 ms. It means that the noise of the signal has reduced to a lower level and the output of lasers has been stable.

For this instrument, each laser was set to be on for 400 ms long in each scanning cycle to avoid higher signal noise. The scanning cycle for eight cantilevers was a little more than 3 s. This period of time meets the requirement of data acquisition for biochemical detection experiments, which always need a few minutes to several hours.

C. Temperature change response

For a bi-material cantilever, the cantilever bends when the temperature changes. This is the reason why the thermal expansion coefficients of different layers are different. The deflection of a rectangular cantilever caused by temperature change can be expressed as²⁷

$$\delta = 3(\alpha_1 - \alpha_2) \left(\frac{n+1}{K} \right) \frac{l^2}{t_2} \Delta T, \quad (1)$$

where δ is the vertical deflection of the cantilever tip, α_1 and α_2 are the thermal expansion coefficients of the two layers of the cantilever, l is the length of the cantilever, ΔT is the change of the ambient temperature of the cantilever, and

$$K = 4 + 6n + 4n^2 + \frac{E_1}{E_2} n^3 + \frac{E_2}{E_1} \frac{1}{n}, \quad (2)$$

$n = t_1/t_2$, where t_1 and t_2 are the thickness and E_1 and E_2 are Young’s modulus of the two layers of the cantilever, respectively. For Au/Si bi-material microcantilever, K can be calculated and will be a constant. As a result, we can figure out that the deflection δ is linearly related to temperature ΔT . The deflections of the cantilevers in one array should be equal when the temperature changes.

In order to verify that the instrument has a consistent response with the cantilevers in an array, a temperature response test was conducted by changing the temperature of the liquid cell where the microcantilever array was placed. The deflection result is shown in Fig. 3(a). The temperature was increased 2.00 °C every 15 min from 24.00 °C to 32.00 °C controlled by the Peltier element. It took about 5 min for the temperature to be stable after the temperature was changed. The cantilevers deflected when the temperature increased, and the deflections remained when the temperature was stable. The cantilever deflection was extracted when the temperature was stable and shown in Fig. 3(b) as a function of temperature. The deflection decreases linearly with the increase of temperature. Linear fit coefficients (adjacent R-square) were greater than 0.99. The deflection is linearly related to the temperature, which is a typical temperature characteristic of the bi-material cantilever. This instrument accurately reproduces this feature. The slopes of the lines in Fig. 3(b) represent the deflections per degree Celsius of eight cantilevers. The relative deviations of the deflection per degree Celsius were no more than 7.6%. The testing result reflects that the instrument responses consistently to deflections of the cantilevers in an array.

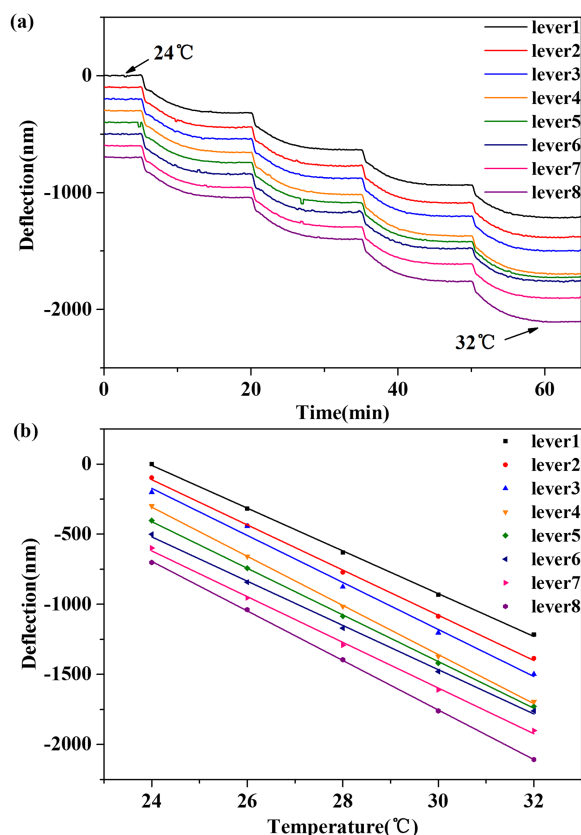


FIG. 3. Temperature change response of the sensor system. (a) Temperature change response curves. Temperature is changed 2.00 °C every 15 min from 24.00 °C to 32.00 °C. For clarity, curves are separated vertically by an interval of 100 nm at 24.00 °C and in order from lever1 to lever8. (b) Linear fitting curves of cantilever deflection as a function of temperature. Linear fit coefficients were greater than 0.99. The relative deviations of the deflection per degree Celsius were no more than 7.6%.

In this paper, the microcantilever array sensor was systematically realized and finely adjusted. Lasers were set on temperature controllers to keep the lasers working at a stable condition. The ends of optical fibers were fixed and sealed in a silicon etching groove with a pitch of 250 μm to ensure one by one correspondence between the lasers and cantilevers. An industrial PLC was used to realize sequential control of lasers, temperature control, data processing, and collection to ensure long-term stability of the sensor system. The temperature of the liquid cell was controlled precisely to avoid noise from external environment temperature change. The signal noise was found to increase when lasers were working at higher frequency, and the laser used here was set to be on for 400 ms in each scanning cycle. Deflection signals of the microcantilever array were found in good alignment.

IV. DETECTION APPLICATION

The gold coated microcantilever array was used to detect mercury ion in aqueous solution and an immune sensor was developed to detect clenbuterol, respectively, to test the performance of the sensor instrument. Mercury is a heavy metal and a highly toxic environmental pollutant.²⁸ Hg(II) can accumulate on the gold surface in aqueous solution.²⁹ When Hg(II) accumulates on the gold surface of the cantilever, the surface stress

of the cantilever will change and cause a bending deformation. The accumulation rate is faster and the final deposition amount is larger at higher concentrations of Hg(II) , causing greater deflections of the cantilevers. Clenbuterol, which is a growth-promoting agent for livestock farming, is banned in China because of serious toxic side effects such as acute poisoning.³⁰ We immobilized the clenbuterol antibody on the gold surface of four cantilevers with microcapillaries to detect clenbuterol, while the other four cantilevers were set as reference.

A. Hg(II) detection

$\text{Hg(NO}_3)_2$ (1 mg/ml, containing 2%-5% HNO_3 , J&K Scientific Ltd., China) was used as original sample solution. HNO_3 (65%-68%, Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) was added to the solution to keep the pH value (6.0) in order to prevent the formation of HgO . The microcantilever array was cleaned and placed in the liquid cell, immersing in aqueous solution. The liquid flow rate was kept 1 $\mu\text{l/s}$ and the temperature of the liquid cell was set at 25.00 ± 0.05 °C.

To examine the consistency of different cantilevers response in an array, we measured the deflection curves of all the cantilevers within an array in response to 10 ng/ml Hg(II) . When stable baselines of the deflection signals were obtained, 10 ng/ml Hg(II) solution was injected to the liquid cell. We defined cantilever bending toward the silicon side as bending down (negative) and toward the gold side as bending up (positive). The deflection result was shown in Fig. 4. All cantilevers bended in the same direction. Accumulation of Hg(II) on the gold layer generated compressive stress and caused negative deflection of the cantilever. Deflections tended to be stable after 1 h since the injection of the Hg(II) solution. Deflections of different cantilevers in the array were very similar. Average deflection of the cantilever array was about -421.5 nm at 80 min, shown by the inset in Fig. 4. There was no big difference observed between the deflections of eight cantilevers. The deflection curves showed good consistency, and it looked like that the consistency of the results was better than that of the results got from a commercial microcantilever array platform (Cantisens, Switzerland).^{31,32}

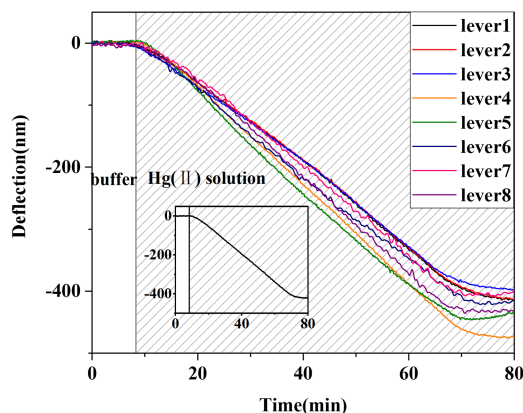


FIG. 4. Microcantilever array deflection curves for detection of 10 ng/ml Hg(II) . The inset graph shows the average deflection and average deflection is about -421.5 nm at 80 min. The shaded area shows the period of the flow of Hg(II) solution.

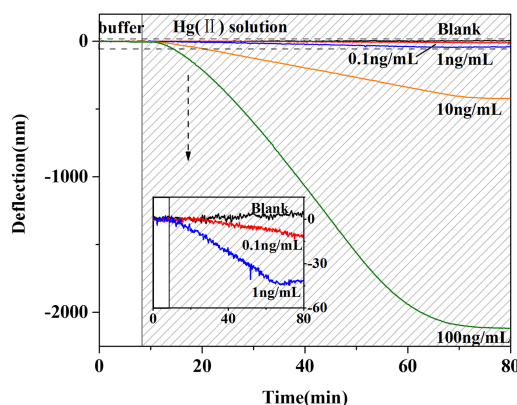


FIG. 5. Average deflection for different concentrations of Hg(II) as a function of time: Blank(0), 0.1, 1, 10, 100 ng/ml, the inset graph is an enlarged display of the dotted area. The shaded area shows the period of the flow of Hg(II) solution.

To verify the quantitative detection ability of the microcantilever array sensor to different concentrations of Hg(II), the sensor was used to detect Hg(II) with the concentration of 0.1, 1, 10, and 100 ng/ml, respectively. New microcantilever arrays were used for different concentrations of Hg(II). Samples were injected into the liquid cell, respectively, when stable baselines of the deflection signals were obtained. Average deflections of different concentrations are shown in Fig. 5. When higher concentration of Hg(II) was detected, the deflection was greater and the deflection curve got a bigger absolute value of the slope at the same time. Apparently, it is because that higher concentration of Hg(II) accumulated more on the gold surface, which caused larger compressive surface stress of the cantilever, causing greater deflection. The final accumulation of Hg(II) was stable in a certain concentration. Average deflections of the cantilevers in an array of different concentrations were about -10.8 , -41.5 , -421.5 , and -2117.0 nm in sequence. A reference test was also taken by adding a buffer to the liquid cell, and no specific deflection was observed.

B. Clenbuterol detection

N-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), ethylenediamine-*N*, *N*, *N'*, *N'*-tetraacetic acid (EDTA), and thioglycolic acid HSCH_2COOH were purchased from Sigma-Aldrich. All other chemicals were analytical grade. The anti-clenbuterol polyclonal antibody and clenbuterol were available from our previous studies.³³ Buffers and solutions used include phosphate-buffered saline (PBS) (0.1M phosphate buffer containing 0.9% sodium chloride, pH 7.5) and PBS with 0.1% (v/v) Tween-20 (PBST).

The microcantilever array was exposed to a freshly prepared piranha solution [$\text{v}(\text{H}_2\text{O}_2) : \text{v}(\text{H}_2\text{SO}_4) = 1:3$] for 5 min, rinsed three times with deionized water, and dried under a gentle stream of nitrogen gas. This microcantilever array was immersed in 1 mM thioglycolic acid solution in ethanol for 12 h at room temperature. Functionally active NHS-esters were obtained by the reaction of thioglycolic acid carboxyl group with a fresh mixture of 0.2M EDC and 0.05M NHS in water

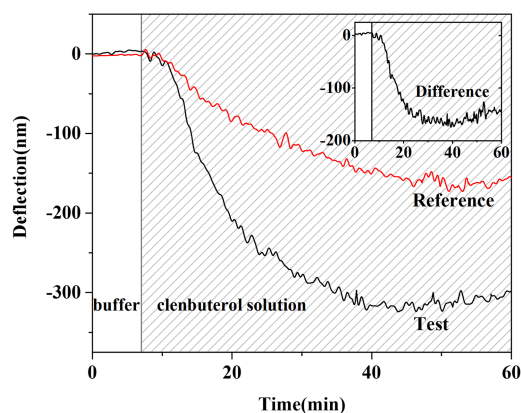


FIG. 6. Average deflection for 200 ng/ml clenbuterol. There is a clear difference between the average deflection of the test cantilever and the reference cantilever. Inset graph shows the average differential signal and the deflection is about -143.0 nm at 60 min. The shaded area shows the period of the flow of clenbuterol solution.

for 30 min. Then, the clenbuterol antibody was immobilized on the microcantilever alternately by using the microcapillary method.³⁴ Four cantilevers in an array were inserted into the microcapillaries filled with the antibody solution for 3 h at room temperature, while the other four were used as reference. At last, the functionalized microcantilever array was washed with PBST three times and fixed in the liquid cell filled with PBS buffer. The temperature of the liquid cell was set at 37.00 ± 0.05 °C and the flow rate was set at $1 \mu\text{l/s}$. PBS solution containing 200 ng/ml clenbuterol was injected into the liquid cell after stable baselines were obtained.

Figure 6 shows the average deflections of test and reference cantilevers. Negative deflections are observed on the test and reference cantilever after the injection of sample. This may be because that nonspecific binding reaction like physical adsorption of clenbuterol happened on the test and reference cantilever surface. There is a clear difference of deflection between the test and reference cantilever. The inset graph shows the differential deflection of about -143.0 nm at 60 min, which should be caused by the specific binding of clenbuterol and the antibody on the cantilever surface.

V. CONCLUSION

We developed a microcantilever array sensor instrument based on the optical lever method. The instrument was realized by utilizing a fiber ribbon coupled to eight lasers and converged into eight microfabricated V-grooves as light sources of the microcantilever array. Precisely etched Si V-grooves and aspherical lens were used to keep one to one correspondence between lasers and microcantilevers. The temperature of the lasers, the liquid cell, and the surrounding environment of the instrument was well controlled to ensure that this sensor instrument provided stable temperature conditions for biochemical detection. The cantilever signal was obtained with a high signal-to-noise ratio by controlling the scanning cycle of the light sources, and the scanning cycle was set to 3–4 s. The signal noise was about 2 nm. The working processes, including temperature control, time control, and data processing, were well controlled by the PLC to make the system work stably for a long time. The linear deflection response of the cantilevers

to temperature change confirmed a consistent response of the instrument. Application experiments were conducted by quantitative detection of mercury ions in aqueous solution, and it showed consistent deflections at the same concentration. Clenbuterol was detected by setting test and reference cantilevers in an array and a stable differential signal was obtained. The microcantilever array sensor instrument shows good stability and practical performance.

ACKNOWLEDGMENTS

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