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An Infrared Radiation based Thermal Biosensor for Enzymatic Biochemical Reactions

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Abstract— **In this paper, a thermal biosensor based on the infrared radiation energy is proposed for calorimetric measurement of biochemical reactions. Having a good structure design combined with MEMS technology as well as employing the Si /SiGe quantum well sensing material with a high TCR and low 1/f noise, the sensor shows potentials to be high sensitive and real-time. The urea enzymatic reaction was tested to verify the performance of sensor, which demonstrates a linear detection range from 0.5mM to 150mM and a relative standard deviation less than 1%. For the sensor fabrication, wafer-level transfer bonding is a key process, which makes the integration of quantum well material and a free standing structure possible. It reduces the heat loss from the sensor to the surrounding environment.**

I. Introduction

Advanced animals usually own the ability to regulate the body temperature due to plenty of exothermic biochemical reactions in the cells. The bio-thermal effects in an organ or body system have been thoroughly studied, whose results are being used in medical examinations [1-2] and disease diagnosis [3-5]. For instant, the allergic reactions in the immune system are often accompanied by feverous symptoms through the body and a long-term symptom of low fever is usually happened for precancerous patients. Apparently, these peculiar exothermic reactions are of great significance, especially in endotherm animals and human beings. Interestingly, few studies are focused on the thermal effects of a single cell which is due to the lack of sensitive detection platforms. Most of biological reactions can only release weak heat energy, and the heat will dissipate into the surrounding water environment quickly, which limits the application of thermal biosensors to some extent. However, as a kind of promising non-destructive detection tools, ultra-sensitive thermal biosensors are still worthy of being studied, which can be used to develop a direct view of cell metabolism.

At present, many thermal biosensors are developed for exothermic biochemical assays [6]. One of them is "enzyme thermistor" based on specific enzymatic reactions. These reactions are able to generate quite large temperature change during the measurement. In fact, all thermal biosensors have to focus on a physiologic range of temperature, which is about 273K to 318K [7]; otherwise, living cells or biomolecules will become disabled at even higher temperature. According to our investigation, it is found that the sensitivity of most thermal biosensors in that critical temperature window is not high enough, though their measuring ranges could extend widely. A long response time of the present thermal biosensors is another point that left for concern, which may cause the difficulties to track temperature changes occurring at faster rates Compared to the other biosensors, thermal biosensor shows a relatively slow development [8-9].

Here, a novel thermal biosensor was designed by Si/SiGe quantum-well materials [10], which can generate sensitive responses to the slight temperature changes at mK level within microseconds. Several biocompatible materials, including SU-8, PDMS and gelatin, were employed during the fabrication of this thermal sensor. Gelatin is a well-known product that derived from the collagen inside skin and bones of animals, which is commonly used as a substrate to culture adherent cells and also a common embedding material for enzymes or living cells in the field of bioengineering. Furthermore, its IR absorbance ratio at the wavelength around 10μm is relative lower than other biocompatible materials. Although gelatin hydrogel is easy to melt in hot water, its endurance is high enough to support the thermal biosensor in a proper measuring range. In the future, the surface of gelatin gel film could be used to adsorb target cells, and then thermal reactions inside the cells could be studied on the spot.

In this study, the surface of thermal sensor was coated by an enzyme-embedding gelatine layer, which forms a catalysis biofilm. Urease, an enzyme that catalyses the hydrolysis of urea in to carbon dioxide and ammonia, was embedded inside the gelatin gel to demonstrate the principle of this thermal biosensor. The reaction occurs as follows:

$$(NH_2)_2 CO + H_2O \rightarrow CO_2 + 2NH_3$$

II. Methods

A. Sensor Design

The device consists of a thermal biosensor chip integrated with the PDMS microfluidic channel cover [11]. The microfluidic channel allows the solutions to be introduced or removed as well as offers exposed circuits a protection from the solution. For the detector itself, working as a thermal sensor, it is expected to present a low conductive heat loss to the substrate. With this factor considered, a suspended structure of sensor is designed as shown in Figure 1(a). In this structure, a trench is introduced which meets the requirement of longitudinal electrical pathway of the applied Si/SiGe quantum well when the conductive pads are all arranged on

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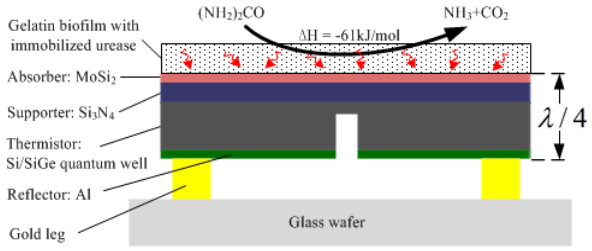
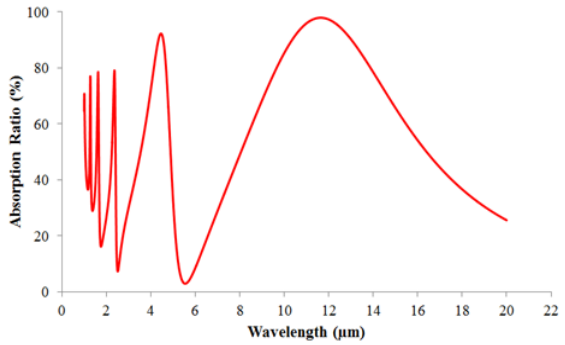
the bottom surface of the sensor. At the same time, it benefits the self-heating reducing as a result of the increased electrical resistance in the thin film.



(a) Sensor structure interacting with the top biofilm through the infrared radiation



(b) Simulation results of the infrared energy absorption ratio at the specific thickness for every layers: $MoSi_2$ (80nm), Si_3N_4 (360nm), Al (150nm)

Figure 1. Sensor's structure with the SU-8 and PDMS unshown and the simulation result for the λ/4 optical resonator

Every object near room temperature presents the thermal radiation corresponding to a high spectral emittance range of infrared light at the wavelength 3-20μm and this infrared radiation energy value will vary with different temperature. The sensor is designed to have a high absorption at the wavelength range 10-13μm, where the gelatin presents a large transmission window for the infrared light. Under the only consideration of normal incidence light, an average of absorption efficiency 95% is obtained through the analytic simulation.

The sensor employs four basic function layers. IR radiation energy absorption is enhanced by a molybdenum silicide ($MoSi_2$) film as well as the bottom aluminum reflector layer, which causes a temperature increase detected by the Si/SiGe quantum well thermistor. Moreover, the silicon nitride is chosen to offer an adequate mechanical strength of the suspended thin film. And its relative high thermal conductivity will not influence the film temperature too much. In the application, the urease is immobilized in the gelatin film and the urea will diffuse into the gelatin biofilm from the above aqueous solution in the PDMS channel.

B. Fabrication Process

In the biosensor, the Si/SiGe quantum well material is deposited in the RPCVD reactor of ASM Epsilon 2000 under the condition of pressure 20Torr and temperature 650°C. The substrates are cleaned using a standard ex-situ cleaning procedure and immediately loaded to the N_2 purged load lock. The gas sources are Si_2H_6 and GeH_4. High-resolution X-ray diffraction is used for the layer profile calibration.

As described above, it usually needs a high temperature to deposit this quantum well thermistor, usually 600-700°C. And if using the MUMPs process to fabricate our biosensor, this high temperature may destroy the beneath aluminum layer and the gold lines on the glass wafer substrate. So in our application, the wafer-level transfer bonding is employed as a promising way to integrate the quantum well sensing material onto the top of the glass substrate having the electrical path.

The fabrication starts with two independent wafers: a glass wafer with the electrical path and a SOI wafer with deposited Si/SiGe quantum well structure. For the glass wafer, SU-8 negative photoresist and electroplating were employed in the fabrication for the conductive legs on the glass wafer. It makes long and thin gold legs possible. After 25μm SU-8 is coated onto it, the gold pads are exposed by lithography and etching. Then the wafer is put into a pre-mixed acidic gold electroplating solution on the market (K-24EA10, pH4.0) at a temperature of 50°C. For the SOI wafer with deposited Si/SiGe quantum well structure, an additional aluminum reflector deposited on to it with a thickness 150μm by sputtering.

Then at this stage, the two wafers are ready to be bonded together using the gold-to-gold ultrasonic bonding. The ultrasonic bonding is a cold joining process which doesn't affect the quantum well material. It directs high-frequency vibrations at two components that are clamped together and no adhesives or consumables are required. Here, a good alignment of these two wafers is necessary. After bonding, the handle and BOX layers of the original SOI wafer are removed by dry etching process. Then the thermistor material is effectively transferred to the glass wafer having the electrical path for signal read-out. The following processes complete the sensor's structure. The Si_3N_4 and $MoSi_2$ layers are deposited by LPCVD and sputtering, respectively. Then a 10um SU-8 is spun onto the wafer again and a cavity above the sensor is created by lithography. At last, a gelatin layer with the immobilized urease is spun to have a contact with the sensor surface.

C. Experiment Set-up

Figure 2 shows the experiment setup that used for the bio-reaction analysis, which verifies the energy transfer from the infrared radiation to thermal heat. The resistance change of the sensing material is detected by the electrical signal processing. In the experiment, the two chambers, one of which is for the reference, were filled with test sample and buffer (without urea) using syringe pumps, respectively. The sensor was electrically wire bonded to the ceramic pads, where the substrate of the sensor prototype was glued to. The electrical signal processing consists of several modules shown in Figure 2. The most important one is the ROC circuit where the operational amplifier AD8269 (Analog Devices™) is applied and the introduced capacity can prevent the self-oscillation.

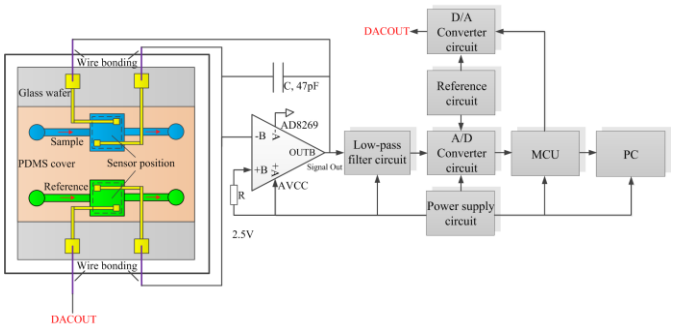


Figure 2. Experiment set-up for sensor testing with urea solution

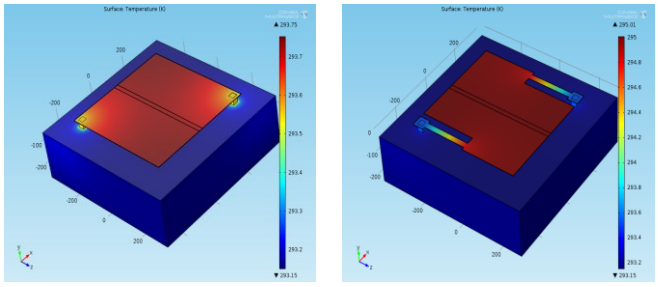
III. Results and Discussions

When judging a sensor's performance, the sensitivity and response time are always the two aspects to be concerned more. In our design, combining with the MEMS technology, the sensing area is 500μm*500μm and the sample can be reduced to several micro liters or even less. This gives a reduced thermal capacity and in turn a fast response speed.

In the application, the most common enzymatic reaction is chosen for the verification. This thermal biosensor can be easily applied to the many specific biochemical reactions for the detail study, even though some of them have a low enthalpy change.

A. FAM Analyzing

A heat transfer analyze was conducted in the Comsol software. It was assumed a uniform and steady heat flux on the surface of the sensor. As a heat flux is related to the concentration of the urea sample, taking the main factors involving enthalpy change and infrared transmittance into consideration, a value of 160W/m^2 is applied to the simulation. Moreover, the influence of SU-8 was neglected, which is acceptable due to its low thermal conductivity and non-contact with the bottom surface of the sensor.



(a) without beam (b)with beam

Figure 3. The comparison simulation results for the sensor structure with and without beam.

Figure 3(a) shows the result of the initial designed structure, which only presents a 600mK difference between the maximum temperature rising and the surrounding environment. In order to optimize the sensor performance, a beam was introduced into the structure (shown in Figure 3(b)) with a length 200μm and width 20μm. These added beams reduce the working area to some extent, but a more uniform temperature distribution on the sensing area is obtained causing most of the temperature drop will occur on the beam. Most important result is that the maximum temperature difference is increased to 1850mK due to the high thermal resistance of introduced beam. And this gives a chance to a sensitive detection of biochemical reaction with low enthalpy change.

Through the dynamic records with the maximum temperature rising and time, a response curve can be easily got. The response time value is taken at the exact time when the temperature rising gets to the 63.2% of the steady one. Using the Matlab to fit the records point and we get a response 30ms and 8ms with the structure with and without the beam, respectively. Both structures show a rapid response time. So, with both the sensitivity and rapidity taken into consideration, introducing the beam to the structure is a good improvement for the sensor performance.

B. Urea Biochemical Reaction

For every urea analysis, the calibration standards were prepared by 2-fold dilution of urea stock solution in 100mM PB buffer, pH 7.0. In order to study the impact on the biosensor by the pH in solutions, two unessential PB buffer (pH 7.5 and pH 6.5) was also prepared. Three calibration standards curve were gained with a replacement of the PB buffer in the tests. All the measurements were carried out by injection of the sample solution into the testing chamber. Once the urea solution filled the testing chamber, the urea .molecules would diffuse into the urease-embedding layer on the bottom and then exothermic reaction started. Si/SiGe thermal sensor would collect the signal of heat radiation generated in the biofilm. After the response of the sensor was read within seconds, the testing chamber should be washed by PB buffer, pH 7.0 immediately in order to preserve the reproducibility of the biosensor.

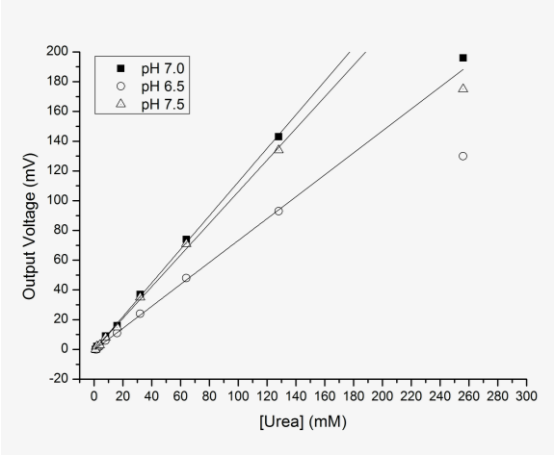


Figure 4. The testing results with different concentration of urea solution and the pH of buffer solution

The results of the output voltage were recorded in the Figure 3 with different concentrations and pH values. From this result, when the concentration of urea solution is smaller than 130mM, the output voltage is linear quite well at a certain pH value. If the concentration comes to the 260mM, it presents a large offset from the linear line. This is due to a saturation of the urease inside the gelatin film. Even more

concentration of urea solution was introduced for the reaction, but no more urease can be used to catalyze the excessive urea. For the biochemical reaction, pH value is a critical factor which will influence the results quite much. The reason is due to the characterization of enzymatic activity. In our test, it shows a best reaction at the pH around 7, which means that the urease is suitable to work in a neutral environment. When the pH is changed to the 7.5, there is no obvious difference with that for pH 7, from which we can conclude that the urease is not so sensitive to the week alkaline environment. However, with a week acid solution, the situation is totally changed. A big difference is observed from that in the neutral environment.

The results of linear detection range from 0.5mM to 150mM and a relative standard deviation less than 1% are comparable with previous reported devices, such as the paper shown in reference [12].

A dynamic range is also achieved when the biosensor is stored in the refrigerator. Every 3 days, the biosensor would be tested by the same sample, 128mM urea solution (pH 7.0) in the same conditions. The stability of the biosensor is over the designed period, see Figure 5. The biofilm showed excellent stability. The preliminary tests indicated that the half-life of an enzyme-embedding biofilm could be more than a month if stored properly. What's more, the sensor can be easily reusable after changing the biofilm.

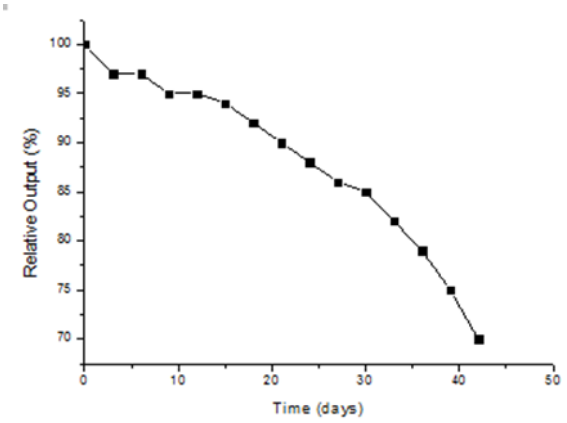


Figure 5. Stability of urease response to 128 mM urea solution using Si/Ge thermal biosensor. The urease-embedding biofilm was made in Day 0 and tested by 128 mM urea solution in 100mM phosphate buffer (pH 7.0) every 3 days. At the testing intervals, the biofilm and thermal sensor were stored in the refrigerator. All the experiment conditions were the same. The testing output in the Day 0 was defined as 100%, and then the other results were normalized by comparing with the data in Day 0.

IV. CONCLUSION

A novel thermal biosensor is designed and tested for the urease measurement. In the paper, an optimization for the sensor is presented for better performance. Differing from the existed thermal biosensor, our designed sensor applies the energy radiation that exists in all the objects that are higher than 0K with the thermistor. With a well selected cover, which is PDMS in our application, the background noise can be reduced as much as possible. What's more, by applying a high resolution read-out circuit, it can be easily to achieve a high sensitive detection with low enthalpy change.

Moreover, with the modern MEMS technology development, a much smaller pixel of this thermal biosensor can be fabricated to study the activity of a single biological cell. It will be worthy to explore the properties of different kinds of cells.

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