Mems Based Immunosensors for Biodetection and Development of Novel Read-Out Methods

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ABSTRACT
Micromechanical cantilevers show great potential as highly sensitive biochemical sensors. Cantilever-based sensing involves the transduction of a biomolecular interaction to a measurable mechanical change in the cantilever resulting from induced surface stresses added mass. In surface stress sensing applications, one side of the cantilever beam is rendered sensitive to a specific target molecule, while the opposing surface is chemically passivated .When target molecules interact with the sensitized surface of the cantilever, the change in surface stress between the sensitized and passivated surfaces results in a measurable mechanical deflection of the cantilever beam.This paper shows micro cantilever based bio detection and different integrated read-out methods and their characterization. We also show that cantilevers have a increased sensitivity by changing their shape and geometry. Recent advances in MEMS promise considerable and realistic potential for the development of innovative and high performance sensing and diagnostic approaches in biomedical field.

1. INTRODUCTION
The diagnostic principle of nanomechanical deflection of the micro cantilever due to adsorption of the antigen on its upper surface is employed for the diagnosis. The deflection of the micro cantilever would be measured in terms of piezoresistive changes by implanting boron at the anchor point. Such a biomicro electromechanical system (BioMEMS) based micro diagnostic kit is highly specific as complementary biochemical interactions take place between antigens and the antibodies against them immobilized on the upper surface of the micro cantilever. The paper discusses the various aspects of the development and production of micro cantilever based sensors. This paper proposes a new micro cantilever design with a rectangular hole at the fixed end of the cantilever that is more sensitive than conventional ones.
Biosensors are electronic devices that convert bimolecular interactions into a measurable signal. The purpose of biosensor is to detect and analyze the unknown biological elements present in a medium. Biosensors have two main elements, a bioreceptor and a transducer. Bioreceptors are target specific and known biomolecular that combine with the target analyte molecules, and generate a unique signal during the reaction. For sensing purpose one surface of the biosensor is functionalized by depositing a sensing layer of known bioreceptor molecules onto it. This biosensitive layer either contains the bioreceptors or the bioreceptors are covalently bonded to it. The most common types of bioreceptors used in biosensing are based on proteins, antibody/antigen or nucleic acid interactions. The transducer element of the biosensor converts the biomolecular reactions between the target and bioreceptor molecules into a measurable signal. The signals can be measured using appropriate detection techniques like electrochemical, optical or mechanical.

2. FEATURES OF CANTILEVER BASED BIOSENSORS AND DIFFERENT READ OUT METHODS
Cantilever-based sensing is a growing research field within micro technology with research groups found around the world [1-10]. The technology offers a method for rapid, label-free, on-line and in-situ detection of specific bio/chemical analytes by detecting the nanomechanical response of a cantilever sensor. Cantilever sensors can be operated either in dynamic mode or static mode.
In the dynamic mode the resonance frequency, f0, of the cantilever is monitored and as masses adsorb onto the structure, the resonance frequency decreases. This change in resonance frequency, Δ*fres*, for a homogeneously distributed adsorbed mass is given by, equation (1).

$$|\Delta f_{res} \approx -f_0 \frac{\Delta m}{2m_0}$$

where Δ*m* is the mass of the adsorbents and *m0* is the initial mass of the cantilever. Most measurements of bio/chemical reactions are performed in liquid, where dynamic mode operation is difficult due to viscous damping. In the static mode it is the surface stress generated when molecules selectively adsorb onto one surface of the cantilever that is measured. The generated surface stress results in a bending of the cantilever, typically in the order of a few 100 nm's. Figure 1 shows this principle schematically.

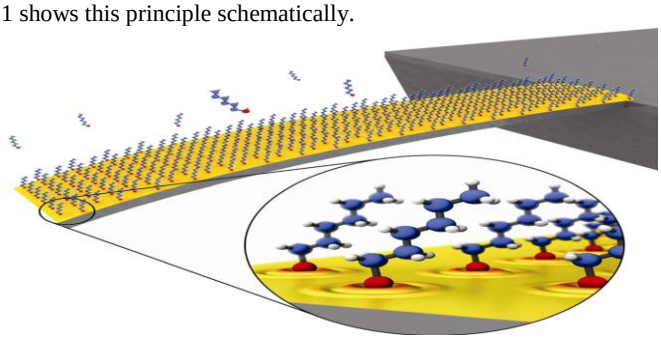


Figure 1. As molecules selectively bind to one surface of the cantilever, the structure is deflected due to the generated surface stress.
The degree of cantilever bending can be related to the amount of analyte present [1]. The deflection, Δz, of a
cantilever with a length-to-width ratio much larger than one operated in static mode is described by equation (2).

$$\Delta z = \frac{3 \cdot \Delta\sigma \cdot (1-\nu) \cdot l^2}{E \cdot t^2}$$

where Δσ is the differential surface stress, ν is the Poisson's ratio and *E* is the Young's modulus of the cantilever material respectively and *l* and *t* are the length and thickness of the cantilever respectively.

Modes of operation and detection methods

Micromechanical cantilever-based sensors can be operated, sometime simultaneously, in two modes: the *static mode* where the bending of the cantilever is measured and the *dynamic mode* where the change in resonance frequency of the cantilever is monitored (see Figure 2).

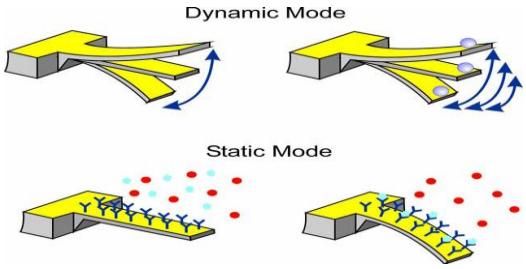


Figure 2 : Schematic drawings of the two possible modes of operation of cantilever-based sensors.

The static mode is sensitive to surface stress changes and temperature fluctuations whereas the dynamic mode is susceptible to mass variations (microbalance). The bending and resonance frequency shifts can be measured with high precision using a variety of methods. The simplest way of measuring cantilever deflection is by optical beam deflection as in most AFM instruments. In the optical beam deflection technique a laser diode is focused at the free end of the cantilever and the reflected laser beam is monitored using a position sensitive photo detector. The typical displacement sensitivity achieved using this technique is on the order of 10-9 m. Its advantages are its simplicity, linear response, and lack of electrical connections. However it suffers some limitations. A calibration is needed in order to obtain the recorded signal in terms of the actual cantilever deflection. Index of refraction changes of the surrounding medium of the cantilever can produce artificial deflection and the technique cannot be used in opaque media such as blood.
Another optical method which can attain better performance is interferometry. When using a fiber optic interferometer, the interference signal from the reflected light off the cleaved end of the fiber optic and off the cantilever surface is a direct measure of the cantilever displacement. Deflection in the range of 10-11 to 10-13 m can be measured [1]. However, this method is confronted with a few technical problems. The fiber must be delicately positioned in proximity of the cantilever and only small displacements can be measured. Another alternative is the
piezoresistance method. Piezoresistivity is the variation in the bulk resistivity under applied stress. When a silicon cantilever is stressed because of its bending, a highly doped region will change resistance in a sensitive way. The variation of cantilever resistance is typically measured with a dc-biased 7 Wheatstone bridge. The advantage of this technique is that the sensor and the detection scheme can be easily integrated into lab-on-a-chip type devices. In addition it is more compatible with large array formats. However, this method possesses electrical connections which need to be protected for experiments performed in liquids and requires current to flow through the cantilever. This results in heat dissipation and thermal drifts which causes parasitic cantilever deflections. Other, less widely used, readout schemes exist such as the capacitive method, piezoelectric method and electron tunneling. More recently, displacement detection methods for nanoscale cantilevers were implemented.

3. FEATURES OF CANTILEVER BASED BIOSENSORS

In biosensing applications sample preparation and molecular labelling of the target analyte is a basic requirement. Labelling aids in easy detection and monitoring of the biomolecules and bioreactions progress. Radioactive and fluorescent dye based labelling agents are commonly used in biosensors. Labelling is however an expensive and time consuming process. Therefore, label-free detection technique is critical in developing rapid, economic and user-friendly biosensors and bioanalytical kits. Cantilever array biosensors use optical detection technique to measure the surface-stress induced deflections in a microcantilever. When the target molecules attach to their functionalized surface, the surface stress distribution on the surface is changed causing deflections in the cantilever

(Figure 1). During adsorption of target molecules onto the functionalized cantilever surface, biochemical reactions occur which reduces the free energy of the cantilever surface. The reduction in free energy of one side of cantilever is balanced by increase in strain energy of the other side, producing deflection in the cantilever. The deflections may be upward or downward depending on the type of molecules involved and are linearly proportional to the target analyte solution concentration. It means that higher deflections manifest higher sensitivity in the cantilever biosensor. Since the induced surface stress strongly depends on the molecular species and its concentration, by measuring the cantilever deflection the attaching species as well as its concentration can be determined.

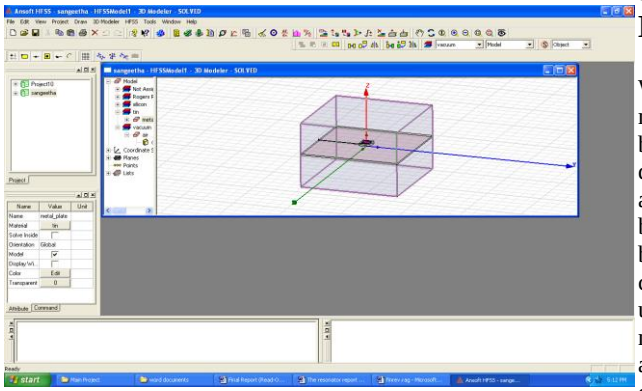
Each biosensor has two primary components: bio-recognition element and transducer. The biorecognition element, such as antibody and phage, is highly specific to the target species . The reaction between the target species and the bio-recognition unit would result in some changes in the physical/chemical properties of the recognition unit. These changes are measured using a transducer. Different types of transducers have been developed and extensively investigated in recent years. One important type of the transducer is the acoustic wave (AW) device, which is an acoustic resonator and works as a mass sensor. That is, the reaction between the bio-recognition component and the target species results in a change in the mass load of the transducer/resonator, which shifts the resonance frequency. Thus, by monitoring the resonance frequency of the AW device, the reaction between the biorecognition unit and the target species, such as captured bacterium cells by antibody/phage, can be determined. An AW device as a transducer used in biosensors is characterized using two critical parameters: mass sensitivity (Sm) and quality merit factor (or Q value).
The mass sensitivity is defined as the shift in resonance frequency due to the attachment of a unit mass, while the Q value reflects the mechanical loss of the devices and characterizes the sharpness of the resonance peak in the

amplitude/phase versus frequency plot. A higher (Sm) means a more sensitive device, while a higher Q value represents a capability to determine a smaller change in resonance frequency (i.e. a higher resolution in determining resonance frequency). Therefore, it is highly desirable for an AW device to have a higher Sm and a larger Q value. Among all AW devices, micro/nano-cantilever exhibits extremely high sensitivity primarily due to its small mass. For example, the detection of a mass as small as 10-18 g using cantilever has been demonstrated. Therefore, a great deal of efforts has been spent on the development of micro/nano-cantilever based biosensors.

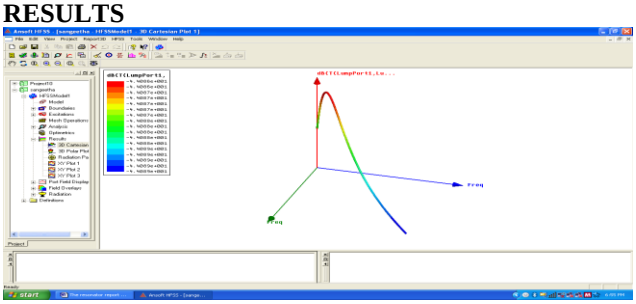
Different types of cantilevers made of different materials have been developed as transducers used in biosensors. In terms of actuating and sensing technologies, all the cantilevers can be classified into two types: passive and active. The passive cantilevers, such as silicon-based cantilevers, require a separated system to actuate the device and usually use a separated optic system to measure/monitor the vibration of the device. On the other hand, the active cantilevers, such as piezoelectric-based cantilevers, can be easily actuated by simply applying a driving field, such as an electric field in the piezoelectric case, and the vibration behavior of the active cantilever can be easily sensed/monitored, such as by measuring impedance in the piezoelectric case. Due to the easiness and availability of the micro/nano-fabrication technology, silicon-based cantilevers are much more widely investigated than others. Additionally, silicon-based cantilevers exhibit a higher Q value than piezoelectric-based cantilevers.

4. SIMULATION AND IMPLEMENTATION OF MICROCANTILEVER SENSOR WITH DIFFERENT READ OUT METHODS

Here we are going to design the transducer/resonator in HFSS (Ansoft) 9.2 version.



RESULTS



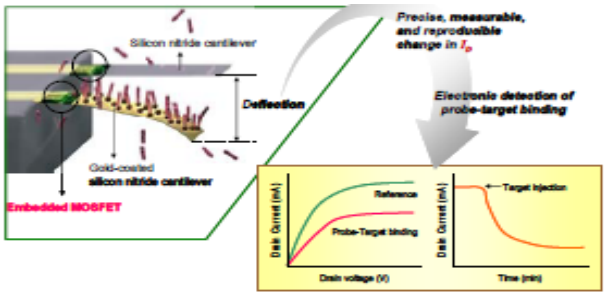
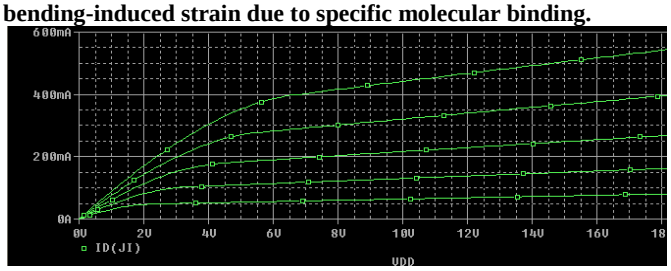


Figure 3 Schematic illustration that embedded MOSFET provides drain current change as the signal due to bending-induced strain due to specific molecular binding.



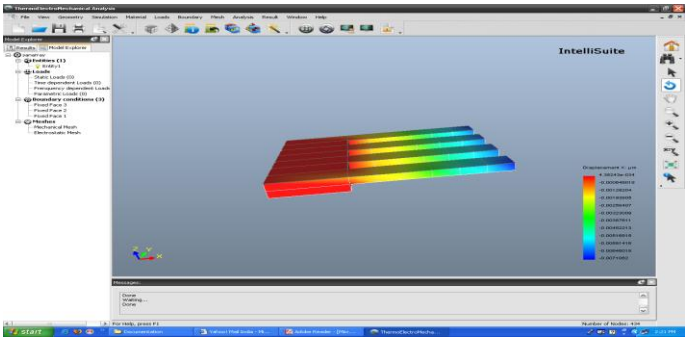
OUTPUT GRAPHS OF SENSORS

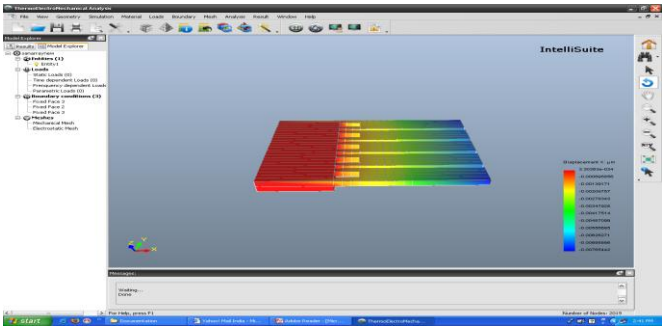
5. SENSITIVITY OF CANTILEVER BIOSENSOR

With the ability of label-free detection and scalability to allow massive parallelization already realized by microcantilever biosensors, the next challenge in cantilever biosensor development lies is achieving the sensitivity in detection range applicable to in vivo analysis. The sensitivity of a cantilever biosensor strongly depends on it ability to convert biochemical interaction into micromechanical motion of the cantilever. The deflections of a cantilever biosensor are usually of the order of few tens to few hundreds of a nanometer. Such extremely low deflections necessitate use of advanced instruments for accurately measuring the deflections. As a consequence, most of the applications of cantilever biosensors are done in laboratories equipped with sophisticated deflection detection and readout techniques.

The detection of analytes in such large dynamic range requires an extremely sensitive cantilever. This paper proposes and analyses a new high sensitive cantilever design that can assay analytes in extremely low concentrations. This paper proposes a new microcantilever design with a rectangular hole at the fixed end of the cantilever that is more sensitive than conventional ones.

The resonant frequency depends on both the material and geometric properties of the cantilever. If we are using silicon cantilevers the reduction in resonant frequency is practically not significant because silicon has excellent mechanical and thermal properties. Due to its high elastic modulus, silicon cantilevers will not be much affected by the external sources of
excitation. Polymer cantilevers in contrast can be significantly affected by the reduction in resonant frequency owing to their low elastic modulus. Therefore the resonant frequency of polymer cantilevers should be increased, which can be achieved by increasing their thickness. The proposed design may not be suitable for polymer cantilevers. Hence, for increasing the sensitivity of polymer cantilevers, instead of changing their shape changing the size is a better option. Thus, based on the above discussions on the bimetallic effects, the large deflection behavior and the interpretations of Equation we can safely conclude that increasing the cantilever thickness is a better way to increase the sensitivity of polymer microcantilevers used in biosensing application. For in vivo detection we need a sensitive biosensor that can assay analytes in large concentration range simultaneously. For such biomedical applications a new array design is proposed (Figure3). The figure shows a comparison between conventional and proposed cantilever eight cantilevers array designs. The conventional array design uses eight cantilevers of uniform cross-section. Proposed array design uses a combination of old and news cantilever designs. Since the proposed cantilevers are nearly twice sensitive than conventional, they can be used effectively in assaying target analytes whose solution concentration is comparatively lower. In both the array designs one cantilever type in each can be assigned as a reference for differential deflection readout, which is a popular mean to eliminate noise in deflection signals. The reference cantilever is made passive by depositing buffer materials onto it, and hence it does not participate in the reaction. Thus, we may conclude that by using an array combination of conventional and proposed cantilevers on the same biochip, a high sensitive biosensor can be designed. Such a sensor can simultaneously detect analytes in extremely large, dynamic concentration range

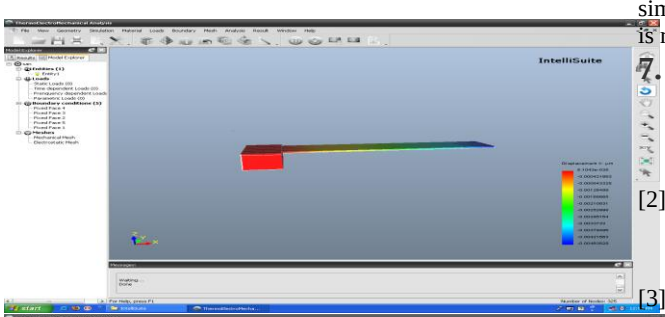


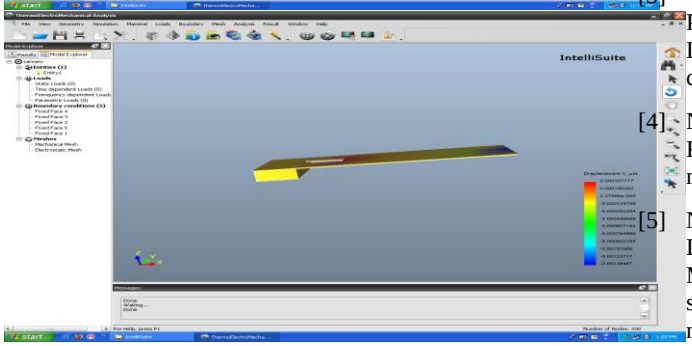


The sensitivity of the device is directly proportional to the length by thickness ratio (L/t) of the microcantilever. Therefore, the longer and thinner the microcantilever is, the greater is the sensitivity of the device.

6. CONCLUSION

Microcantilever array biosensors are becoming increasingly popular in label-free, realtime and simultaneous detection and monitoring of various chemical and biochemical target analytes. The deflections in microcantilever biosensors lie between few tens to few hundreds of a nanometer, which necessitate sophisticated and expensive readout techniques. The ultimate goal of the microcantilever biosensor design and development is to make them sensitive enough to be used in medical applications where accurate, realtime and simultaneous analysis of various clinically important analytes is required.





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