

Micro-cantilever Sensors for Detection of Pesticide Contents in the Water-table of Malwa Region in Punjab

Gurleen Kaur, Ravinder Singh Sawhney, Rajan Vohra

Department of Electronics Technology, Guru Nanak Dev University, Amritsar

Email: gurleen_gnps2000@yahoo.com; Sawhney_gndu@hotmail.com; sargam_111@yahoo.co.in

Abstract- Biosensors based on microcantilevers have become a promising tool for directly detecting biomolecular interactions with great accuracy. Microcantilevers translate molecular recognition of biomolecules into nanomechanical motion that is commonly coupled to an optical or piezoresistive read-out detector system. Biosensors based on cantilevers are a good example of how nanotechnology and biotechnology can go together. High-throughput platforms using arrays of cantilevers have been developed for simultaneous measurement and read-out of hundreds of samples. As a result, many interesting applications have been developed and the first sensor platforms are getting commercialized. In this paper, we propose the application of Microcantilever sensor for the detection of pesticide contents in the water table of the Malwa region in Punjab (infamous as the cancer belt of the region) due to environmental toxicity following the excessive use of pesticides and fertilizers under the disguise of Green Revolution. The results concluded here can be applied in general to the various parts of India, where use of the pesticides is excessive and that is causing the adulteration of the water table.

Keyword— Microcantilever, Biosensor, NEMS, Biomolecular interaction

I. INTRODUCTION

Microcantilevers are micromechanical beams that are anchored at one end, such as diving spring boards those can be readily fabricated on silicon wafers and other materials. Their typical dimensions are approximately 100 microns long, 20 microns wide and 1 micron thick. The microcantilever sensors are physical sensors that respond to surface stress changes due to chemical or biological processes [1]. When fabricated with very small force constants, these can measure forces and stresses with extremely high sensitivity. The very small force constant (less than 0.1 N/m) of a cantilever allows detection of surface stress variation due to the adsorption (or specific surface-receptor interaction) of molecules [2]. Adsorption of molecules on one of the surfaces of the typically bimaterial cantilevers (silicon or silicon nitride cantilevers with a thin gold layer on one side) results in a differential surface stress due to adsorption-induced forces, which manifests as a deflection [3]. In addition to cantilever bending, the resonance frequency of the cantilever can vary due to mass loading. These two signals, adsorption-induced cantilever bending when adsorption is confined to one side of the cantilever and adsorption-induced frequency change due to mass loading, can be monitored simultaneously [2-7] Capacitance, piezoresistance and resonance frequency are among the sensing principles depending upon the mechanical properties of the device.

Micromachined cantilevers were first used as force probes in Atomic Force Microscopy (AFM). Their extreme sensitivity to several environmental factors, such as noise, temperature, humidity and pressure was immediately evident. In 1994, research teams in Oak Ridge National Laboratory and IBM, converted the mechanism causing interference into a platform for a novel family of biosensors. By measuring the change in resonance frequency, microcantilevers were shown to be sensitive to mass changes, with a better yield than piezoelectric gravimetric conventional sensors. The laboratories with MFA instruments displayed a substantial interest in cantilevers as a new platform for a variety of chemical and physical biosensors.

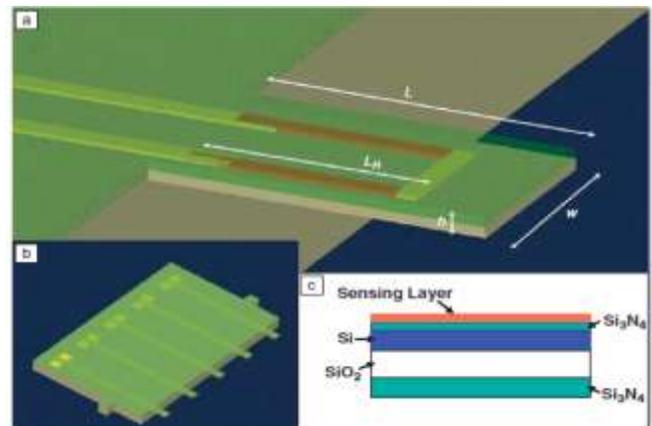


Figure1. (a) Schematic diagram of a microcantilever indicating dimensions length, L , resistor length, LR , width, w , and thickness, h . (b) An array of piezoresistive cantilevers. (c) Cross-sectional diagram through the layers of the microcantilever sensors [3]

With recent advancements in technology, microcantilever sensors have found wider applications in the field of medicine, specifically for the screening of diseases, detection of point mutations, blood glucose monitoring and detection of chemical and biological warfare agents. These sensors have several advantages over the conventional analytical techniques in terms of high sensitivity, low cost, simple procedure, low analyte requirement (in μl), non-hazardous procedures and quick response. Moreover, the technology has been developed in the last few years for the fabrication and use of nanocantilevers for sensing applications, thereby giving rise to nanoelectromechanical systems (NEMS) [8]. This development has increased the sensitivity limit up to the extent that researchers can now visualize the counting of molecules.

Microcantilever sensors are most promising for microbiosensors and nanobiosensors. This new class of highly sensitivity biosensors can perform local, high resolution and label-free molecular recognition. The microcantilevers translate the molecular recognition of biomolecules into nanomechanical motion [9] (from a few nm to hundreds of nm), which is commonly coupled to an optical or piezoresistive read-out system [10,11]. Research in this new type of sensor is growing exponentially after the landmark paper of Fritz et al. in 2000, in which the ability of microcantilever sensors is shown in discerning single-base variations in DNA strands without using fluorescent labels. Shortly afterwards, microcantilever sensors were also shown to work in DNA hybridization [11] and detection of proteins involved in cancer [12] and other diseases [13] with increased accuracy, as well as in environmental sciences [14]. Cantilever sensors have also been used for detecting chemicals, such as volatile compounds [15], warfare pathogens [16], explosives [17], and glucose [18], and ionic species, such as calcium ions [19].

II. THEORY OF OPERATION

A microcantilever is a device that can act as a physical, chemical or biological sensor by detecting changes in cantilever bending or vibrational frequency. It is the miniaturized counterpart of a diving board that moves up and down at a regular interval. This movement changes when a specific mass of analyte is specifically adsorbed on its surface similar to the change when a person steps onto the diving board. But microcantilevers are a million times smaller than the diving board having dimensions in microns and different shapes as shown in figure 2.

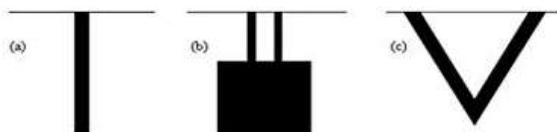


Figure 2: Different types of microcantilevers (top view)
(a) Rectangular (b) Double-legged (c) Triangular [19]

The immobilization of molecules on the cantilever surface is required for its use as a nanomechanical sensor (Figures 3 and 4). The selection of the molecule depends on the intended application. This principle applies whether if the molecule to be detected (analyte) will be presented in a solid, liquid or a gas phase. The immobilized molecules provide the cantilever with specificity for the analyte. The specific molecular interactions taking place at the flexible surface of cantilever increase surface tension forcing the cantilever to bend [1].

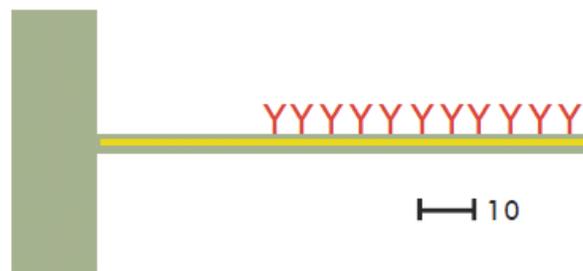


Figure 3. Cantilever (with integrated piezoresistor) without detection.

This type of surface tension induced by molecular interactions is not generally observed on the surface of common materials. The cantilever senses the tension and bends in response to the free energy changes taking place at its surface.

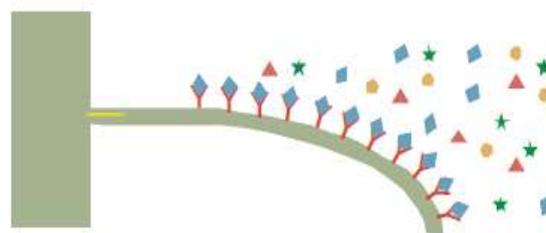


Figure 4. Cantilever with detection. Presence of molecules immobilized at the surface.

Molecules adsorbed on a microcantilever cause vibrational frequency changes and deflection of the microcantilever. Viscosity, density, and flow rate can be measured by detecting changes in the vibrational frequency.

Another way of detecting molecular adsorption is by measuring deflection of the cantilever due to adsorption stress on just one side of the cantilever. Depending on the nature of chemical bonding of the molecule, the deflection can be up or down. Biochips with mechanical detection systems commonly use microcantilever bi-material (e.g. Au-Si) beams as sensing elements. The Au side is usually coated with a certain receptor. Upon the binding of the analyte (e.g. biological molecules, such as proteins or biological agents) with the receptor, the receptor surface is either tensioned or relieved. This causes the microcantilever to deflect, usually in nanometers, which can be measured using optical techniques. The deflection is proportional to the analyte concentration. The concept has been employed in screening certain diseases

such as cancer and detecting specific chemical and biological warfare agents.

III. MECHANICAL PROPERTIES OF CANTILEVERS

The basic mechanical parameters of a cantilever are the spring constant and the resonance frequency. The spring constant k is the proportionality factor between applied force, F and the resulting bending of the cantilever, z . This relation is called Hooke's law.

$$F = -kz$$

The spring constant yields the stiffness of the cantilever. For a rectangular cantilever of length l , the spring constant can be written as

$$k = \frac{3.E.I}{l^3}$$

where E is the Young's modulus and I is the moment of inertia. A typical spring constant for a stress sensitive cantilever is in the range of 1 mN/m to 1 N/m. The resonance frequency f_{res} for a simple rectangular cantilever can be expressed as

$$f = \frac{0.162\sqrt{E} \cdot h^3 \cdot W}{\sqrt{\rho} \cdot l^2}$$

where ρ is the mass density, h and w denotes the height and the width of the cantilever respectively. The moment of inertia for a rectangular cantilever can be written as

$$I = \frac{w \cdot h^3}{12}$$

A simpler expression for the resonance frequency can be written as a function of the spring constant as

$$f = \frac{0.32\sqrt{k}}{\sqrt{m}}$$

where mass, $m = \rho \cdot h \cdot l \cdot w$. The relation shows that the resonance frequency increases as a function of increasing spring constant and of decreasing cantilever mass. [20]

IV. MODALITIES OF CANTILEVER DEFLECTION-BASED SENSING

A uniform surface stress acting on an isotropic material increases (in the case of compressive stress) or decreases (in

case of tensile stress) the surface area as shown in figure 4. If this stress is not compensated at the opposite side of a thin plate or beam, the whole structure will bend. Between the areas of compressive stress and tensile stress, there is a neutral plane which is not deformed. Due to bending, a force F is acting at a distance of x in the neutral plane results in a bending moment $M = F \cdot x$. Therefore, the radius of curvature R is given by:

$$1/R = d^2z/dx^2 = M/EI$$

where E is the apparent Young's modulus and I is the moment of inertia given by the following equation for rectangular beams

$$I = \frac{bh^3}{12}$$

The change in the surface stress at one side of the beam will cause static bending, and the bending moment can be calculated as:

$$M = \frac{\Delta\sigma bh}{2}$$

$\Delta\sigma = \sigma_1 - \sigma_2$ is the differential surface stress with σ_1 and σ_2 as surface stress at the upper and lower side of the cantilever respectively (figure 5). Inserting these values of I and M in the first equation yields Stoney's formula

$$\frac{1}{R} = \frac{6(1-\nu)\Delta\sigma}{Eh^2}$$

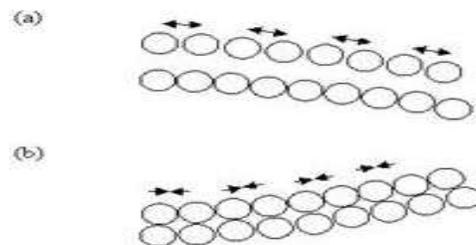


Fig.5 Bending of a cantilever beam in response to compressive and tensile stresses. (a) Compressive surface stress due to repulsion between the biomolecules leads to downward/negative deflection of the cantilever beam. (b) Tensile surface stress due to attraction between molecules leads to upward/positive deflection of the cantilever beam.

Changes in surface stress can be the result of adsorption process or electrostatic interactions between charged molecules on the surface as well as changes in the surface hydrophobicity and conformational changes of the adsorbed molecules. In addition to surface stress-induced bending, the volume expansion of bimaterial cantilevers can result in a

static bending. A bimaterial cantilever undergoes bending due to gas adsorption if the volume expansion coefficients of the two materials are different.

V. APPLICATIONS

The surface of the cantilever should be coated with a layer of detector molecules that can react with the analyte and detect biochemical reactions at its surface. This novel detection technique based on cantilevers is extremely sensitive. Cantilever based biosensors have enormous potential, especially in the field of biochemical analyses. Fast and simple biochemical detectors based on this detection method can be constructed. Studies of simple molecular interactions would also be feasible due to the exquisite mechanical sensitivity of micro - cantilever (Figure 6). The technology of cantilever biosensors is applicable to a number of specific tasks [21]:

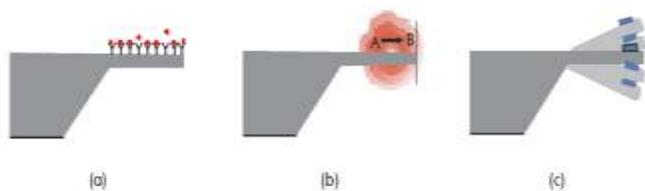


Figure 6: Application of cantilever to the detection of superficial changes (a) tension (b) temperature and (c) mass [21]

1. Life Sciences: For studying the bases of the interaction among biological molecules. To develop novel analyses based on this platform with a potential applicability to portable devices.
2. *In vitro* diagnostics: To develop faster, more sensitive and label free methods (no tracers are needed for final identification) to analyze chemical or biological samples.
3. Drug discovery: To study the interactions between small molecules and their specific receptors. To conduct multiplexed analyses increasing the parallelism and contents of the results.
4. Fresh water control: To detect heavy metal ions in fresh water. To develop assays that could be useful to implement a decentralized system to monitor the quality of the resins, or other chemicals used in the fresh water distribution system [22].

VI. ADVANTAGES

Microcantilever based sensors have enormous potential for the detection of various analytes in gaseous, vacuum and liquid medium. They have aroused considerable interest because of their high specificity, high sensitivity, simplicity, low cost, low analyte requirement (in μl), non-hazardous procedure with fewer steps, quick response and low power requirement.

Substances at trace levels are currently detected by various techniques like high performance liquid chromatography (HPLC), thin layer chromatography (TLC), gas chromatography (GC), gas liquid chromatography (GLC) etc. However, these techniques are complex, time consuming, costly and require bulky instrumentation.

Also sample preparation is a prolonged complex procedure and requires skilled personnel. But the microcantilever-based sensors can detect trace amounts of substances in parts-per-billion (ppb) and parts-per-trillion (ppt). They translate biomolecular recognition into nanomechanical bending of the microcantilever [23]. Intermolecular forces arising from the adsorption of analyte molecules onto the microcantilever induce surface stress, directly resulting in nanomechanical bending of the microcantilever.

VII. PROPOSAL

Punjab state Health Minister Laxmi Kanta Chawla had admitted during Assembly Session on March 7, 2010 that between 2001 and 2009, the total number of cancer patients detected in the rural areas numbered 23,427, of which 16,730 died. Excessive use of pesticides, chemical fertilizers and uranium are being incriminated for making the environment and food chain highly toxic. The surface water as well as ground water has been grossly contaminated by the above mentioned sources of toxicity. The Malwa area of Punjab has emerged as the epicenter of the disease and has come to be known as the cancer belt of Punjab.

A study undertaken by an NGO has indicated that drinking water being supplied particularly in the Malwa belt is source of a cocktail of toxins as it was a combination of pesticides, nitrates, heavy metals and fluoride. Possibly this cocktail was causing the whole spectrum of ill health in Punjab including Cancer [24]. Uranium has also been found in the water samples beyond the permissible limits in groundwater in the Malwa region. Uranium alone or along with other components of the toxic cocktail may be causing mental retardation, physical deformities and neurological problems among children in Punjab and surrounding regions [20].

Keeping the above scenario in mind, there is an urgent need for the sensitive detection of the abnormalities in the soil content. The use of specially fabricated microcantilevers can serve our purpose here. Ultrasensitive microcantilevers can act as precise sensors if we measure their nanomechanical responses in different environments. Microcantilevers can be used to detect the pH of the soil and hence obtain its salinity. It has been spotted that when an intelligent hydrogel is applied to the surface, to detect the deflection of a microcantilever, it swells at variable pH levels. The hydrogel was patterned onto microcantilevers of various sizes using UV photolithography. When the cantilevers were soaked in different buffers, the swelling hydrogel induced surface stress

and therefore the bending phenomenon. The deflection was evident, and differed accordingly at various pH levels [25].

Heavy-metal ions and ions in general have also been studied. Ji et al. used thiol-derivatized calixarene and crown-ether macrocycle-functionalized cantilevers to detect Cs²⁺ ions in the range 10¹¹–10⁷ M and K⁺ in the 10⁴ M range [26]. Following the same grounds amount of Uranium can be detected in the ground water of Malwa region using micromechanical sensors.

Other functionalization schemes have shown that cantilevers were able to detect, with great accuracy and selectivity, different ions, such as CrO₄²⁻ [27], Ca²⁺ [17] or Pb²⁺ [28]. So excessive fertilizer contents like NO₃⁻ and PO₄⁻ can be checked in soils by application of cantilever sensor technology. Nanomechanical cantilever sensors can be the most sensitive devices for pesticide detection using immunoreactions due to the tiny reaction area (100 nm²) compared with another label-free biosensors such as the surface plasmon resonance biosensor (mm²) and the quartz crystal microbalance (cm²). Cantilever based assays for pesticide detection has been reported [29,30]. The stress induced by the binding of a pesticide residue BAM (2,6 dichlorobenzamide) immobilized on a cantilever surface to anti-BAM antibody is measured using with four gold-coated cantilevers and piezo resistive.

Development of nanomechanical biosensors for detection of the pesticide DDT has also been cited. Detection of the organochlorine insecticide compound dichlorodiphenyltrichloroethane (DDT) by measuring the nanometer-scale bending of a microcantilever produced by differential surface stress. A synthetic hapten of the pesticide conjugated with bovine serum albumin (BSA) was covalently immobilised on the gold-coated side of the cantilever by using thiol self assembled monolayers [31]. The immobilisation process is characterised by monitoring the cantilever deflection in realtime. Then specific detection is achieved by exposing the cantilever to a solution of a specific monoclonal antibody to the DDT hapten derivative. The specific binding of the antibodies on the cantilever sensitised side is measured with nanomolar sensitivity. Direct detection is proved by performing competitive assays, in which the cantilever is exposed to a mixed solution of the monoclonal antibody and DDT [32].

SUMMARY

In this review article, we have provided an overview of most technical aspects of the new microcantilever-based sensors which is a promising technology and emerging as a suitable solution for important problems. Improvements in reproducibility and sensitivity, and integration of microfluidics and detection systems are the main aims of current research.

Microcantilevers have got potential applications in every field of science ranging from physical and chemical sensing to biological disease diagnosis. The major advantages of employing microcantilevers as sensing mechanisms over the conventional sensors include their high sensitivity, low cost, low analyte requirement (in µl), non-hazardous procedure with fewer steps (obviating the need for labels), quick response and low power requirement.

The technology holds the key to the next generation of highly sensitive sensors. To sum up, microcantilever-based biosensors comprise a continually growing novel technology and, because of their great capabilities, offer an alternative to current biosensor technologies. Cantilevers will play an essential role in the immediate future of nanobiotechnology.

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Gurleen Kaur is a student of B.tech in Department of Electronics Technology, Guru Nanak Dev University, Amritsar. She is an all rounder and a bright student. She has an outstanding academic career since school times. She represented India in Asia Region Space Settlement Design Competition (ARSSDC), NASA as team leader in 2008 and obtained a gold in National Aerospace Olympiad held at Nagpur in 2009. Though she has been studying in B.Tech Vth Semester, She has already a few publications to her credit.



Prof. Ravinder Singh Sawhney has been working as faculty with the Department of Electronics Technology, Guru Nanak Dev University, Amritsar. He has teaching experience of more than 15 years to both post-graduate and under graduate engineering students. He has more than 35 publications to his credit in various International journals as well as International and National conferences. He has membership of many

International and National Computer Societies. His key areas of interest in the field of Data Communication are routing through WLANs, MANETS, Mesh Networks, MPLS, Ant Colony Optimization, Particle Swarm Optimization and Different Encryption Techniques. He has been on the board of many journals as reviewer. Currently, he is working in the field of modelling and simulation of charge transport through various molecules and designing of various molecular junctions for future Nanoelectronic Devices.



Er. Rajan Vohra has been working as Asstt. Professor in the Department of Electronics Technology, Guru Nanak Dev University, Amritsar . He has experience of two years of teaching post-graduate as well as undergraduate engineering students. He has a keen interest in data communication, especially various applications based on Wireless Local Area Networks and published more than 15 papers in various International journals and International & National conferences. He has been nominated for the finals for the award of young scientist during 15th Punjab science Congress. He has membership of various International and national societies like IACSIT,IAOE,CSTA etc.