Nanomechanical Sensor Platform Based On Piezo-Resistive Cantilevers

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ABSTRACT

This paper presents Cantion's cantilever-based bio/chemical sensor. The cantilevers have integrated readout and can be used both in liquids and gases. The sensing principle is based on a cantilever bending due to a change in surface stress. Such surface-stress change is obtained during molecular interaction which has made it possible to measure DNA hybridization, antigen-antibody interactions etc. This paper presents Cantion's technology and instruments and furthermore discusses different types of applications realized on the platform. Finally, the perspective of using Cantion's cantilever sensor technology for the next generation handheld application is discussed.

Keywords: cantilever, sensor, nanomechanical, label-free, bio/chemical

1 INTRODUCTION

The cantilever-based bio/chemical sensing principle was discovered by T. Thundat *et al*. [1] in 1994 where they found that AFM cantilevers coated with gold would deflect when exposed to for example mercury due to a change in surface stress. They suggested that this could be used as a novel type of sensitive chemical sensor. Since then, a lot of different applications have been demonstrated on the cantilever platform, such as: DNA hybridization [2], antibody-antigen reaction [3] and bacteria detection [4]. Because the sensor technology is based on AFM most of the published work has been based on an optical readout scheme.

Cantion's technology is based on nanomechanical cantilevers as bio/chemical sensors with integrated readout. Which, in contrast to the optical readout scheme known from AFM, makes it possible to make a complete sensor solution with a very small footprint.

Cantion's cantilevers are 100 µm long, 50 µm wide and 0.5 µm thick. The upper side of the cantilever can be coated with a detector layer. This layer has the ability to recognize and interact with molecules of interest. When molecules bind to the detector layer, a change in the surface stress is induced, see figure 1. This effect is usually not observed when the detector layer is placed on a solid surface. However, on the very small and flexible cantilevers this induces a deflection of the cantilever in the order of a few nanometers. The cantilever deflection is picked-up in the integrated piezo-resistor, which then changes its electrical resistance. Thereby the molecular interaction is transduced through a nanomechanical sensor into an electrical signal.

Cantion's sensor can be operated both in liquids and gases.

The generated surface stress on the cantilever is not due to a change in mass but due to the molecular interactions. For example, if the molecules on the surface are electrically charged they will repel each other and thereby bend the cantilever downwards. Other general surface stress generating mechanism are steric hindrance, entropical changes etc. This actually means that the cantilever sensor is able to use many different types of detector layers such as polymers, self assembled monolayers, DNA, proteins etc.

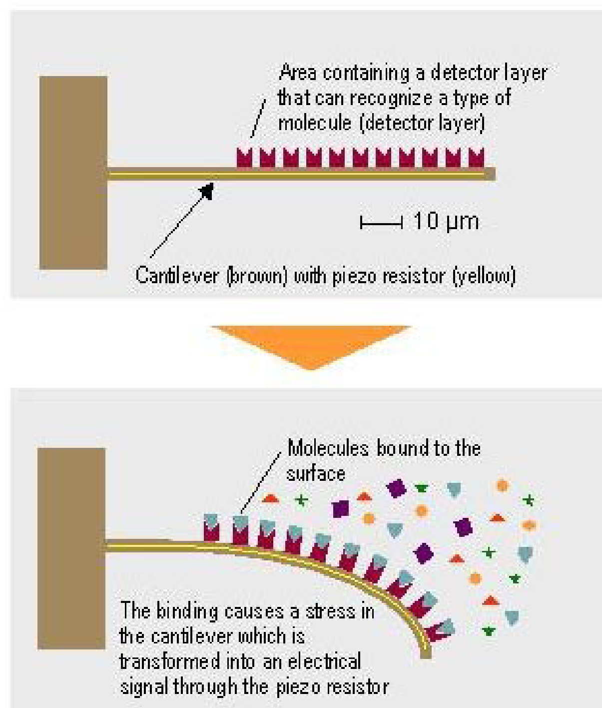

Figure 1: The basic principle for the surface stress sensitive piezoresistive cantilever

The nanomechanical cantilever sensor is by nature sensitive to many different parameters, such as temperature, mechanical vibrations, electrical fields etc. Using a

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differential readout method these effects can be significantly reduced. The differential configuration consists of two cantilevers, one as a measurement cantilever and one as the reference cantilever.

The signal from the two cantilevers can be subtracted in a Wheatstone bridge configuration such that only the differential signal is measured. In order to further minimize the parameters from the environment, Cantion has integrated arrays of cantilevers into a micro fluid handling system. Thermal gradients are thereby significantly reduced compared to larger systems. Furthermore, the laminar flow obtained in micro fluid systems eliminates noise sources from turbulences, which would be a significant noise factor for the cantilevers.

2 INSTRUMENTS

In order to perform reliable and reproducible experiments, Cantion offer an product portfolio consisting of 3 main products:

2.1 Canti™Chip 4

Cantion's Canti™Chip 4 is designed for sensitive detection of surface stress caused by molecular interactions on the cantilever surface. The chip has 4 cantilevers placed in a micro channel. The SEM micrograph in figure 2 shows the chip where 3 of the cantilevers are visible. The chip is packaged unto a printed circuit board for easy handling. The Canti™Chip 4 is optimized for measurement in both gases and liquids and is designed as an open platform, which allows the user to functionalize each cantilever individually.

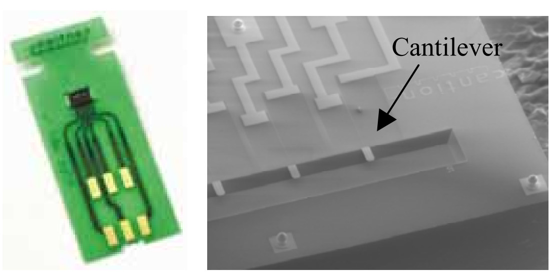

Figure 2: Optical image of chip placed on a PCB board (Canti™Chip 4) and SEM micrograph of the cantilevers

2.2 Canti™Spot

The cantilevers are functionalized using an inkjet printing principle. This is done by aligning each cantilever under a dispenser head whereafter about 100 pl of a given reagent is dispensed on the cantilever. The dispensed volume covers the topside of the cantilever only. The cantilever surface, for example coated with gold, reacts with the reagent and thereby functionalizes the cantilever.

Cantion has developed a research platform for the reagent dispensing, called Canti™Spot. The instrument is shown in figure 3 and consists of a table where the Canti™Chip 4 is placed. By using the xyz microposition system it is possible to align each cantilever underneath the dispenser head.

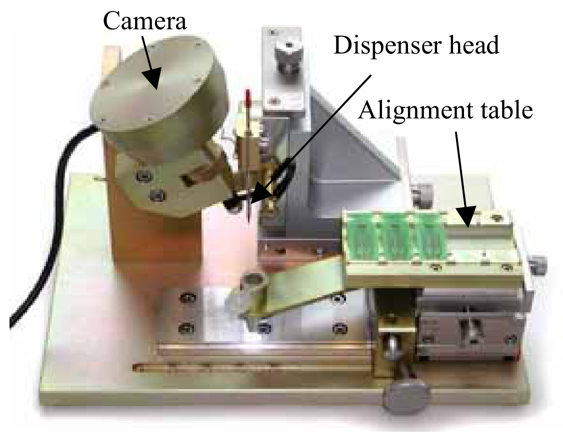

Figure 3: Canti™Spot instrument for functionalizing each cantilever individually. The dispenser head delivers 100 pL of reagent on each cantilever

2.3 Canti™Lab 4

After the Canti™Chip 4 has been functionalized it is ready for performing the experiment. For that purpose Cantion has developed a highly flexible laboratory platform called Canti™Lab 4, see figure 4. The instrument both included the fluid handling system and the electrical readout from the Canti™Chip 4.

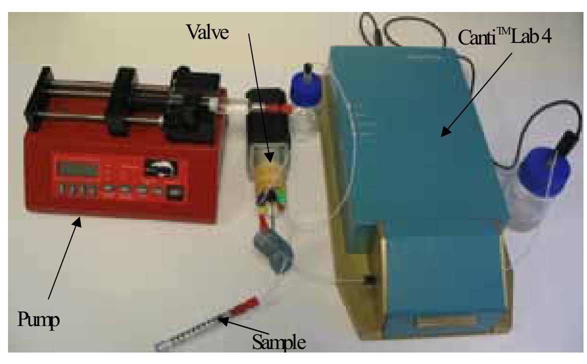

Figure 4: Canti™Lab 4 is a highly flexible laboratory equipment optimized for easy and reliable assay development on the Canti™Chip 4

The sealed interaction cell obtained when the Canti™Chip 4 is inserted in the Canti™Lab 4 has a volume of about 500 nL. This small volume decreases the required sample, which for example is important if the sample of interest is expensive. The small interaction cell furthermore allows laminar flow. The Canti™Lab 4 also features an external liquid system that consists of a programmable pump and valve. Finally, the instrument allows both differential and absolute signals from the cantilever to be measured simultaneously.

3 PROVEN TECHNOLOGY

Different types of assays have been tested on Cantion's sensor platform and a couple of these are discussed in order to demonstrate the potential of the technology.

3.1 Antibody-antigen assay

The first example is an antibody-antigen assay. Usually, such an assay is made by ELISA or similar where a labeled secondary antibody is required. We demonstrate that label-free detection is possible on Cantion's sensor platform.

C-reactive protein (CRP) is an acute phase protein, which is found in the human blood at very low concentrations (<0.1 mg/l). However, if a patient suffers from a bacterial infection the CRP level will increase dramatically in concentration up to 300 mg/l.

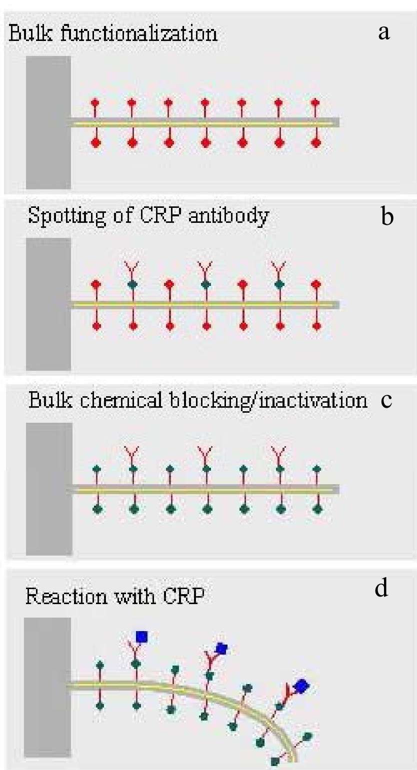

Figure 5: Schematic of CRP experiment. a) activation of silicon nitride, b) functionalization of cantilever by inkjet dispensing, c) blocking of active sites, d) CRP experiment in Canti^{TM}Lab 4

In order to obtain covalently bound CRP-antibodies to the cantilever surface an activation step of the inert silicon nitride surface is required. Eletrophilic groups were obtained on the cantilever surface by an activation procedure developed by Cantion. Hereafter, the Canti^{TM}Spot was used for functionalizaton of the cantilever with CRP antibodies. The backside of the measurement cantilever and the complete reference cantilever was then inactivated by a blocking agent.

The functionalized Canti^{TM}Chip 4 was inserted in the Canti^{TM}Lab 4. First, a stable base line was obtained by flowing buffer through the system. After 500 sec the valve was opened and the buffer + CRP is introduced.

Different concentration of CRP was measured by regenerating the Canti^{TM}Chip 4 after each experiment. In figure 6 three different concentration curves are shown: 0, 5, 100 mg/l. It can be seen that the amplitude of the cantilever signal increases as a function of concentration. The selectivity of the assay was investigated by introducing buffer + 100 mg/l BSA. This did not give rise to any signal (curve was similar to 0 mg/l).

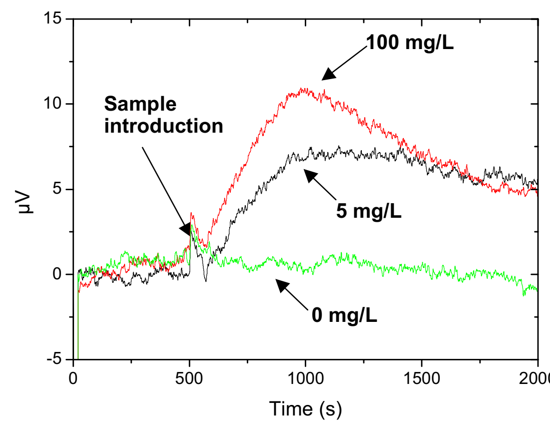

Figure 6: The graph shows the concentration dependent signal. The signal monitored is the change in resistance of the piezo-resistor which is directly proportional to the cantilever bending.

3.2 DNA assay

Another interesting assay developed on Cantion's platform is DNA hybridization. In this case, the cantilever surfaces were coated by gold to facilitate immobilization of thiol-modified oligonucleotides on the surface. In this experiment we used one type of 12-mer oligo on the measurement cantilever and another 12-mer oligo on the reference cantilever. The oligoes were dispensed on to the cantilevers by the use of the Canti^{TM}Spot.

After a stable baseline in a buffer solution was obtained, the valve was opened and buffer + 1 μM of DNA complementary to that on the measurement cantilever was introduced. In figure 7 hybridization curves from 3 different chips is shown. As seen from the graph, the signals are reproducible in terms of amplitude and the required time in order to reach steady-state. A non-complementary DNA strand was also introduced to chip 3 and as seen from the figure this did not give rise to any signals.

3.3 Other types of assays

L. Pinnaduwage *et al.* [5] from Oak Ridge National Laboratory (ORNL) has used Cantion's technology for development of a highly sensitive plastic explosive sensor in ambient air. They were able to obtain a sensitivity of sub-ppt, which is far better than achieved with competing technologies. The explosive detection project on Cantion's platform was so successful that it won an R&D 100 award in 2004.

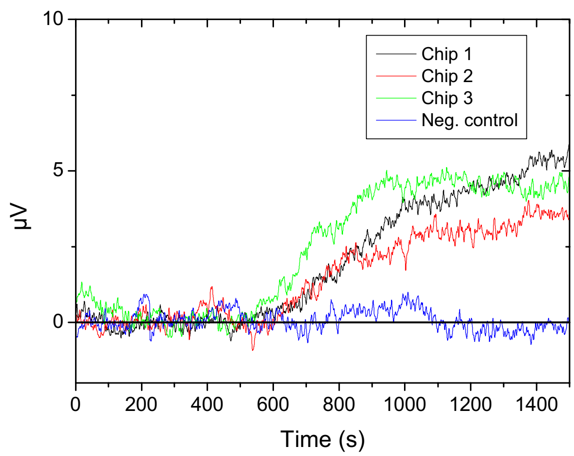

Figure 7: DNA hybridization experiments on 3 different chips. It can be seen that the signals are very reproducible. More signals from other chips showed the same trend.

R. Mukhopadhyay *et al.* [6] from Aarhus University developed a sensitive SNP in DNA assay using Cantion's technology.

Furthermore, Cantion's sensor platform has been used in various other applications, such as alcohol detection, enzyme-substrate detection and chemical warfare agent detection.

4 PERSPECTIVES

The electrical readout principle used in Cantion's sensors makes the technology very robust in different environments and the small sensor and readout footprint makes it ideal for handheld applications. Cantion has developed a prototype of such a handheld device for detection of various gases, see figure 8. The very versatile detection principle used as a handheld device addresses different applications such as detection of explosives, bio/chemical warfare agents, medical point-of-care and food quality control.

Figure 8: Prototype of handheld device based on Cantion's cantilever technology for gas sensing applications.

5 CONCLUSIONS

The paper have presented Cantion's cantilever based bio/chemical sensor with integrated readout. The cantilever sensor is a true label-free detection platform where different types of assaya have already been proven, such as DNA hybridization and antibody-antigen detection in liquid and explosives detection in gas. Due to the electrical readout it is further possible to minimize the sensor platform such that it becomes ideal for handheld applications.

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