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Advanced Biosensing Using Micromechanical Cantilever Arrays

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1. Introduction

During the last few years, a series of new detection methods in the field of biosensors have been developed. Biosensors are analytical devices that combine a biologically sensitive element with a physical or chemical transducer to selectively and quantitatively detect the presence of specific compounds in a given external environment.

These new biosensor devices allow sensitive, fast, and real-time measurements. The interaction of biomolecules with the biosensor interface can be investigated by transduction of the signal into a magnetic ***(1)***, an impedance ***(2)***, or a nanomechanical ***(3)*** signal. In the field of nanomechanical transduction, a promising area is the use of cantilever arrays for biomolecular recognition of nucleic acids and proteins. One of the advantages of the cantilever array detection is the possibility to detect interacting compounds without the need of introducing an optically detectable label on the binding partners. For biomolecule detection, the liquid phase is the preferred one but it has been shown that the cantilever array technique is also very appropriate foruse as a sensor for stress ***(4)***, heat ***(5)***, and mass ***(6)***. Recent experiments showed that this technique could also be applied as an artificial nose for analyte vapors (e.g., flavors) in the gas phase ***(7)***.

2. Nanomechanical Cantilever as Detectors

The principle of detection is based on the functionalization of the complete cantilever surface with a layer that is sensitive to the compound to be investigated. The detection is feasible in different media (e.g., liquids or gas phase). The interaction of the analyte with the sensitive layer is transducted into a

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static deflection by inducing stress on one surface of the cantilever as the result of denser packing of the molecules ***(8)*** or a frequency shift in case of dynamic detection mode ***(9)*** as a result of changes in mass.

3. Overview of the Two Detection Modes

3.1. Static Mode

In static mode detection, the deflection of the individual cantilever depends on the stress induced by the binding reaction of the specific compounds to the interface. The interface has to be activated in an asymmetrical manner, as shown in **Fig. 1**. Most often one of the cantilever surfaces is coated with a metallic layer (e.g., gold) by vacuum deposition techniques and subsequently activated by binding a receptor molecule directly via a thiol group to the interface (e.g., thiol-modified DNA oligonucleotides) or, as in case of protein recognition, by activating the fresh gold interface with a self-assembling bifunctional bioreactive alky-thiol molecule to which the protein moiety is covalently coupled ***(10)***.

The radius R of the curvature of the cantilever is given by Stoney's law ***(11)***:

$$\sigma = Et^2_{\text{cant}}[6R(1-\gamma)]^{-1} \quad (1)$$

where σ is the stress, γ is the Poisson ratio, E Young's modulus, and t_{cant} the thickness of cantilever. The thickness of the lever is an important parameter that can be varied to increase or decrease the sensitivity of the device. By reducing the thickness of the cantilever a larger deflection is achieved. Reducing the thickness by factor '2' increases the bending signal due to stress at the interface by factor '4'. The interaction of the ligand with the receptor molecule has to occur immediately on the interface. No flexible linking of the receptor molecule is allowed as a result of the fact that the induced stress will be diminished. The receptor molecules should be presented in a tightly packed manner on the interface to interact with the substances to be analyzed.

3.2. Dynamic Mode

In the case of dynamic mode detection, the resonance frequency of the individual cantilever, which has to be excited, depends on the mass. The binding reaction of the analyte to the interfaces increases the mass, and the resonance frequency is normally decreased. In **Fig. 2**, the scheme of dynamic cantilever detection is shown.

The cantilever is excited by a piezo element. The change in mass (Δm) during the experiment as the result of an uptake of interacting biomolecules induces a change in the resonance frequency of the cantilever, which can be described by the following formula:

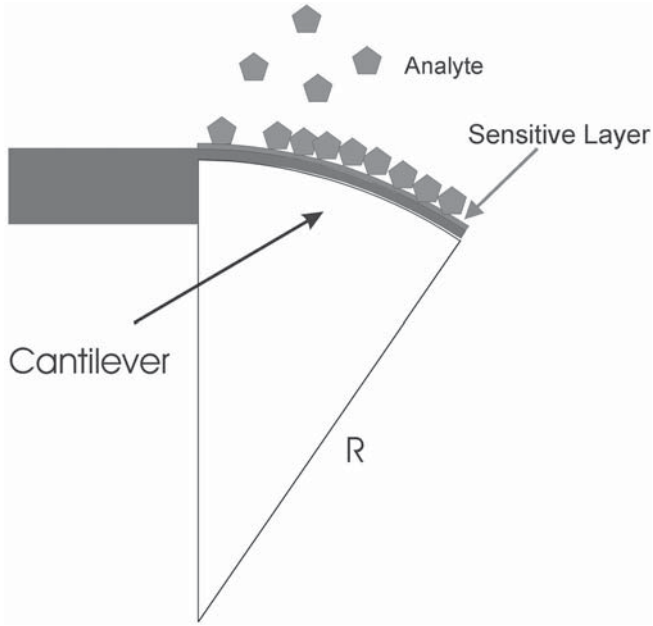

Fig. 1. Interaction of the analyte (light gray pentagons) with the sensitive layer induces a stress on the interface and bends the cantilever (note the asymmetric coating of the individual cantilever surface).

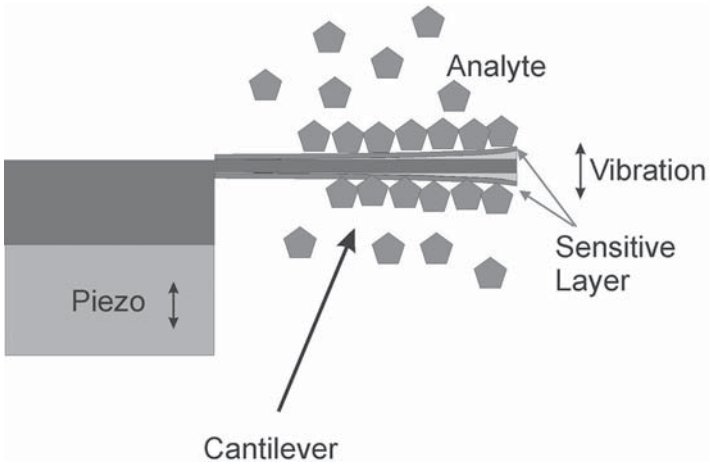

Fig. 2. Interaction of analyte (light gray pentagons) with sensitive layers induces a change in the resonance frequency of the cantilever.

$$\Delta m = k(4n\pi^2)^{-1}(f_1^{-2} - f_0^{-2}) \quad (2)$$

where the resonance frequency before and during the experiment are f_0 and f_1, k is the spring constant of cantilever, and n is a factor dependent of the geometry of the cantilever. The uptake of mass as a result of specifically interacting molecules is doubled in this manner, and the cantilever does not respond to temperature changes via a bimetallic effect. Additionally, the preparation involves fewer steps as in the case of the static detection mode ***(5)***.

4. Setups

At the Institute of Physics at the University of Basel, Basel, Switzlerland, in collaboration with the IBM Research Laboratory Zurich, we developed cantilever array setups both for static and dynamic mode operation in liquids and in the gas phase.

The principal part of the setup is an array of eight cantilevers produced by classic lithography technology with wet etching. A typical picture of such a cantilever array is shown in **Fig. 3**. The structure of an array is composed of eight cantilevers with a length of 500 µm, a width of 100 µm, and a pitch of 250 µm from lever to lever. The etching process provides cantilever thickness ranging from 250 nm to 7 µm adapted for the individual application (i.e., static or dynamic mode).

The cantilever deflection or motion detection is provided by a classic laser beam deflection optical detection for both the static and dynamic mode set up as shown in **Fig. 4**.

The laser source is an array of eight vertical-cavity surface-emitting lasers (VCESLs; 760 nm wavelength, 250 µm pitch), and position detection obtained through a linear position-sensitive detector). The array is mounted in a cell useable for gas or liquid phase measurement.

A scheme showing the setup is displayed in **Fig. 5**. The operation of the instrument is fully automatic and during the time course of a few hours up to eight different samples can be probed using the automatic fluid delivery. The instrumental noise of the static setup lies in the subnanometer range and the dynamic setup is able to detect mass changes in the order of picograms.

The key advantages of cantilever arrays are the possibility of *in situ* reference and the simultaneous detection of different substances. The *in situ* reference is needed to avoid the thermomechanical noise, especially in fluid-phase detection. Changes in refractive index when the buffer changes will also contribute to a so-called virtual motion of the cantilever. As visible in **Fig. 6**, only the real motion, which is the difference in between the cantilevers on the same chip, is originating from the specific biomolecular interaction.

In **Fig. 7A**, a raw signal of the cantilever array is displayed. Because there will always be instrumental or thermal drift, the differential signal detection is mandatory. **Figure 7** shows an experiment with a set of three cantilevers (thickness 500 nm).

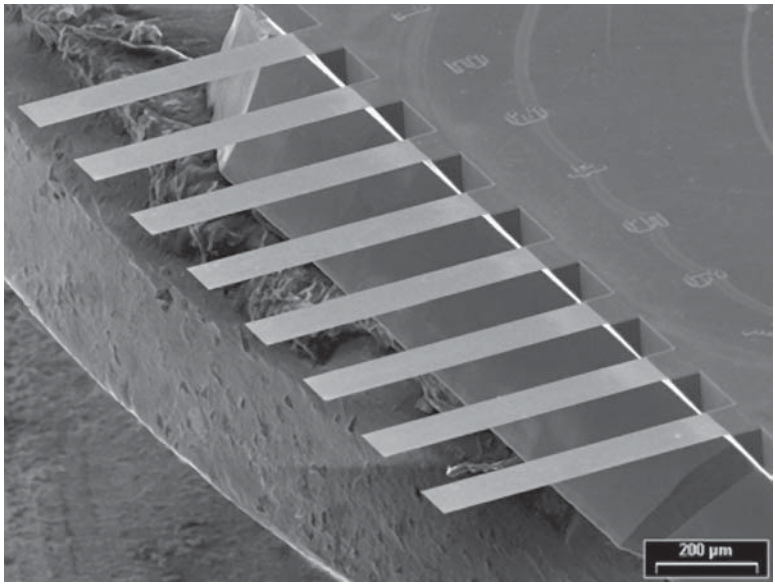

Fig. 3. Scanning electron micrograph of an array of eight cantilevers with individual thicknesses of 500 nm.

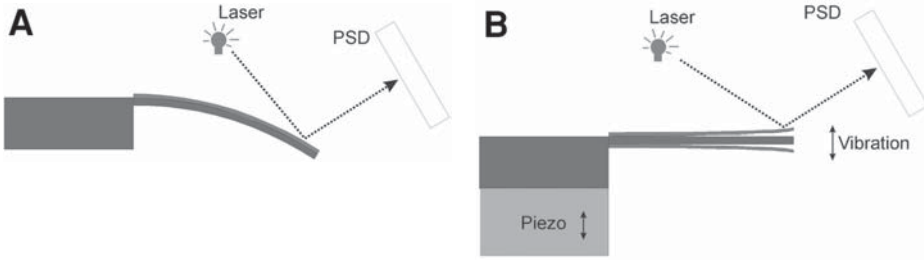

Fig. 4. Detection of average cantilever position using a multiple laser source vertical-cavity surface-emitting laser and a position-sensitive device. **(A)** Static mode; **(B)** dynamic mode.

In this experiment we used two reference cantilevers with different coatings and one specific biorecognition cantilever. By specifically binding biomolecules the cantilever is bending downwards due to stress generated on its surface. As visible in **Fig. 7B**, the differential signal lacks any external influences except for the specific biomolecular interaction, which induces a differential signal of approx 90 nm relative to the *in situ* reference. The experiment is reversible and can be repeated using different concentrations of analytes. In a recent work we presented data that allow the extraction of the

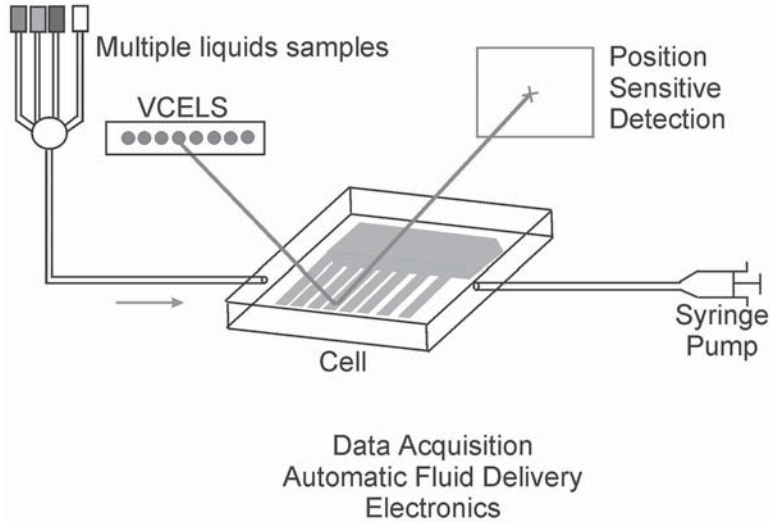

Fig. 5. General structure of cantilever array setups for gas/liquid samples.

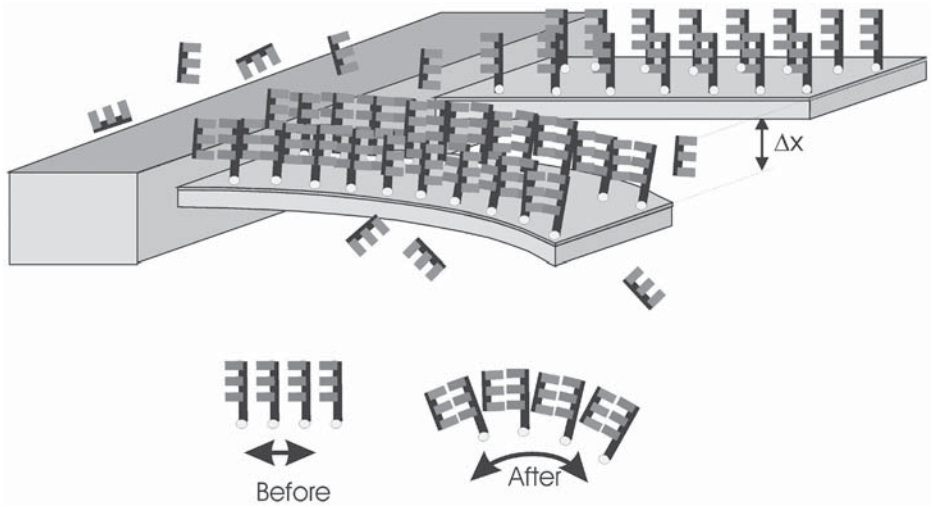

Fig. 6. Static detection of biomolecular interaction. The cantilevers have to be equilibrated before the biomolecule of interest is injected. Because of the specific interaction with the biomolecules (light gray) on the cantilever shown in front, stress builds up that deflects the individual cantilever specifically.

thermodynamics of the interacting biomolecules (i.e., DNA; **ref.** ***12***). Deflection signals as small as a few nanometers are easily detected. Currently, the detection limit in static experiments lies in the range of nanomolar concentra-

tions ***(12)*** but can be significantly lowered in the future by using cantilever arrays in the range of 250–500 nm of thickness.

Great care has to be taken in the selection of the internal reference lever. In the case of DNA detection, an oligonucleotide is chosen that displayed a sequence that does not induce crosstalk binding reactions with the sequences to be detected. Coating with thin layers of titanium and gold using vacuum deposition modifies one side of the cantilever array. Onto this metallic interface, a thiol-modified oligonucleotide self-assembles in a high-density layer. Complementary and unknown oligonucleotide sequences are then injected and the specific interaction is directly visible within minutes. Stress at the interface is built up because of a higher density of packing (*see* **Fig. 6**). In protein detection, a protection of the asymmetrically coated cantilever has to be considered ***(13)***. Preparation of protein-detecting cantilevers is a multistep procedure and requires surface chemistry knowledge. The side opposite to the biomolecular-modified side is generally protected by a polyethyleneglycol layer. The bioreference surface can be coated by using unspecifically interacting proteins (e.g., bovine serum albumin). In protein detection experiments, larger fluctuations of the cantilevers are observed (e.g., **Fig. 7**) than in the ssDNA–ssDNA experiments. A possible interpretation of this difference might be that it is caused by the proteins absorbing light within the visible spectrum and therefore inducing some local changes in the index of refraction. We always measure specific signals within minutes without problems. Normally, some drift of few tens of nanometers is observed in the complete set of cantilevers during the time course of the experiment, even though temperatures of the instruments are stabilized within ±0.05°C. However, these effects are completely eliminated by using a differential read out on the very same cantilever array.

Cantilever arrays are already employed as detectors in both static and dynamic modes ***(8,9)***. Recent articles show the potential for detection of DNA hybridization ***(3,12)***, cell capture, or toxin detection ***(1)***. Integrating cantilever arrays into microfluidic channels will significantly reduce the amount of sample required ***(14)***. Attempts have been made to get data from single-cantilever experiments for DNA ***(15)*** or antibody–antigen reactions ***(16)*** or from a two-cantilever setups using different stiffnesses for the individual cantilevers ***(17)***. We would like to point out that these approaches have serious drawbacks. Information extracted from these experiments, which often last multiple hours, cannot exclude unspecific drift of any kind ***(18)***.

The signal in these experiments is interpreted as specificity on the biomolecular level but no correlation from one lever to the next is applicable if only one lever is used at a time. In the second approach cantilevers with different stiffnesses are used to monitor the nanometer motions. Because the individual cantilever used shows a difference of factor four in terms of stiffness,

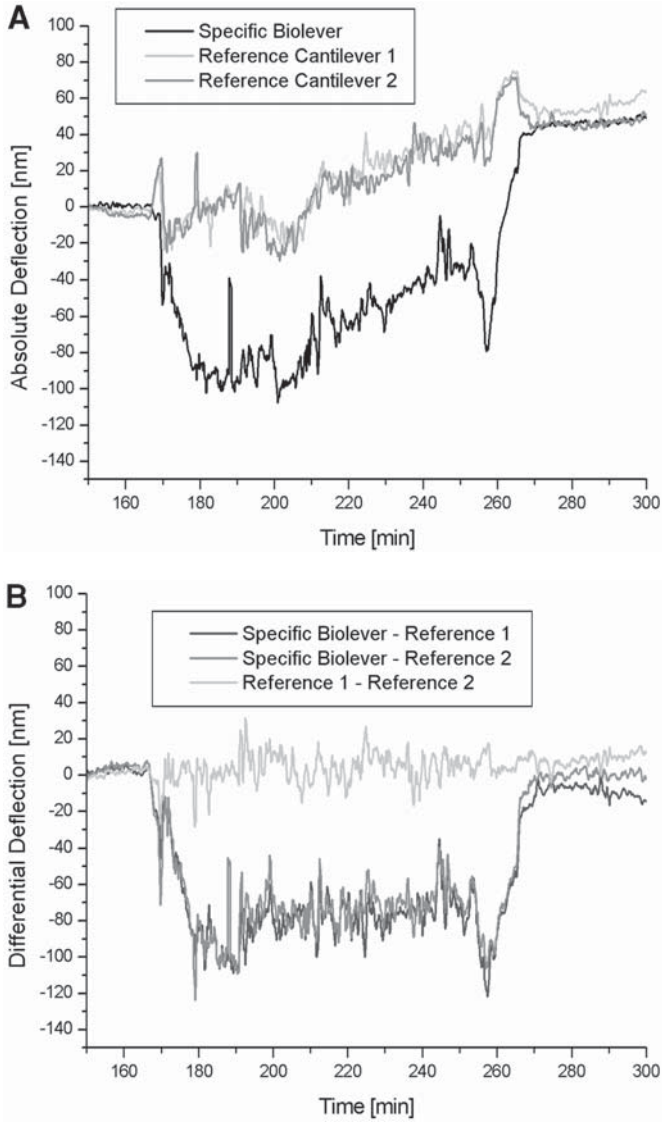

Fig. 7. **(A)** Raw data of a three-lever bioarray experiment. Two shades of gray indicate the motion of the reference cantilevers. In black color, the motion of the biologically specific cantilever is displayed. Upon injection of interacting biomolecules (approx 170 min) turbulences of the liquid cause all levers to undergo some motion, which is stabilized immediately when the flow is stopped (approx 180 min). The specific binding signal quickly builds up and remains stable. The interaction is fully reversible and can be broken by shifting the equilibrium of the binding reaction by injecting pure buffer solution (approx 260 min) into the fluid chamber. Over the course of 2–3 h, we

the response, which originates from a specific interaction, is difficult to extract. The sensitivity of this approach is hampered by the differences in stiffness, which are directly correlated to the thickness of the cantilever used (*see* Eq. 1). An interaction of the biomolecule with the stiffer reference cantilever might not be detectable if the stress signal lies within the thermal noise of that lever.

5. Conclusion

The cantilever array technology explores a wide area of applications; all biomolecular interactions are in principle able to be experimentally detected using cantilever array as long as mass change or surface stress is induced by the specific interaction. A few applications so far demonstrate promising results in the field of biological detection. The cantilever-based sensor platform might fill the gap between the sensitive but costly and relatively slow analytical instrumentation (e.g., mass-spectroscopy, high-performance liquid chromatography, surface plasmon resonance [SPR]) and the chip technologies (for example, gene-arrays) with their advantage of easy multiplexing capabilities, albeit with their need for fluorescence labeling and restriction to higher molecular-weight compounds like proteins and nucleic acids thus far.

In comparison with the methods just described, the cantilever technology is cheap, fast, sensitive, and applicable to a broad range of compounds. The lack of multiplexing could be overcome by the application of large cantilever arrays with >1000 cantilevers per chip. Projects are now underway to introduce commercial platforms providing arrays of eight cantilevers to applications in the liquid or gas phases. A critical point for future development in this field will be the access to the cantilevers arrays similar to that in the normal field biological applications using single-cantilever scanning force microscopy. At the moment there are no biological experiments published that use dynamic mode detection. We believe, however, that its ease of preparation (symmetrically as pointed out) and reduced sensitivity to environmental changes, makes this technology a strong candidate as the instrumental approach of choice for the future biological detection using cantilever arrays.

Fig. 7. *(continued)* regularly see a drift of the complete temperature-stabilized cantilever arrays on the order of tens of nanometers. **(B)** Differential data of the experimental set of Fig. **(A)**. In light grey color, the difference between the two reference cantilevers is shown. Except for some small motions, no differential bending is observed, whereas in the dark gray and black the difference of the specifically reacting cantilever with respect to the reference cantilevers is show. As shown after approx 260 min, pure buffer solution is injected and the differential signal collapses to values close to the starting point were no interacting biomolecules were present in the experiment.

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