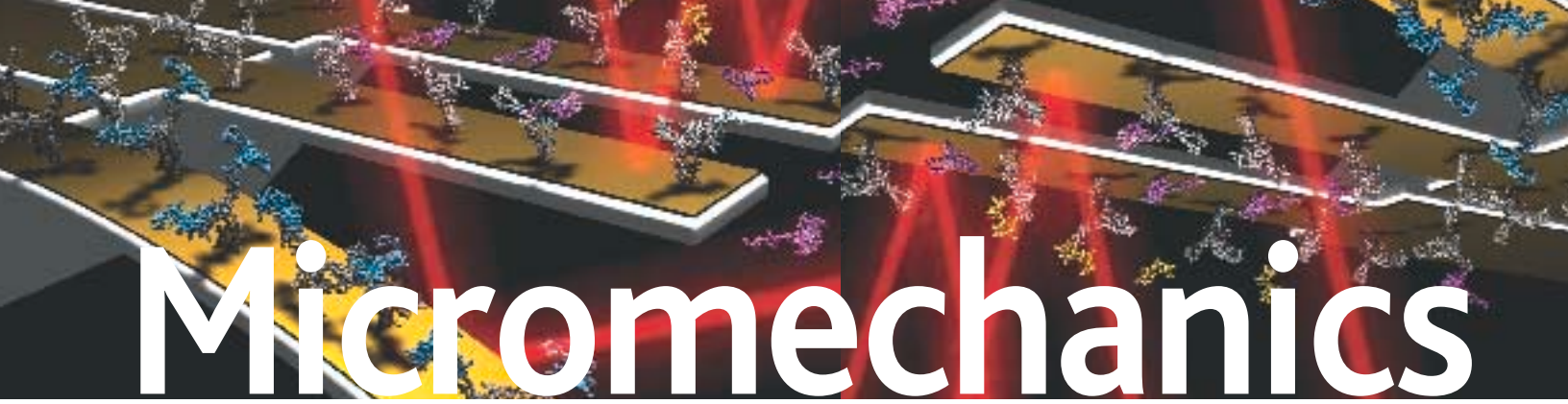

Micromechanics senses biomolecules

by Roberto Raiteri, Massimo Grattarola, and Rüdiger Berger

How can microelectromechanical systems (MEMS) experts support molecular biologists in studying DNA hybridization? Cantilever-based devices are an example of how a 'simple' sensor can be tailored by microfabrication techniques and used to achieve an unprecedented performance. We review fascinating experiments, which use different mechanical transduction principles for detecting and analyzing small quantities of materials. The principles of these experiments allow biologists to study surface biochemistry on a nano-scale and offer new, exciting opportunities in developing microscopic biomedical analysis systems with unique characteristics.

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Image above shows detail from this month's cover. (Courtesy of the University of California-Berkeley. Credit: Kenneth Hsu.)

Cantilever sensors rely on relatively well known and simple transduction principles, and have attracted the interest of many researchers. This is, at least in part, because of the merging of silicon microfabrication techniques and surface functionalization biochemistry, together with the development of multi-cantilever sensing methods offering new opportunities in physical and (bio)chemical sensing.

Interest in microfabricated cantilevers has grown since the development of the atomic force microscope (AFM)[1] in 1986. AFM and related scanning probe microscopy (SPM) techniques allow direct measurement of specific interactions between surfaces at the molecular scale. AFM measures the tiny forces acting on a sharp tip, which is mounted at the end of a long and thin microscopic beam fixed to a support at the opposite end, i.e. a cantilever. The force on the tip bends the cantilever, which acts as a force transducer (Fig. 2a).

The dissemination and application of SPM, both in research labs and industry, is thanks in part to the ease with which inexpensive* force probes with integrated sharp tips can be fabricated in a reproducible way. By shrinking the cantilever structure to a microscopic size one obtains both a low spring constant (i.e. high sensitivity to applied forces or stresses) and a high resonant frequency, for fast response times and high immunity to external mechanical noise. Miniaturization and mass production are achieved by taking advantage of the batch silicon micro-machining techniques developed for integrated circuit (IC) process technology[2,3]. Silicon, silicon oxide or nitride cantilevers are commercially

* Commercial prices for standard AFM probes lay in the $2-20/probe range.

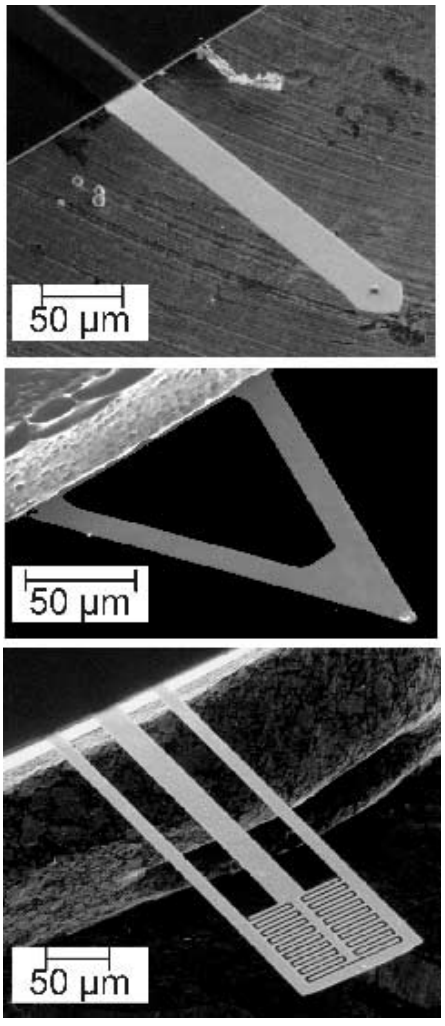


Fig. 1 Silicon, silicon oxide or silicon nitride cantilevers are commercially available with different shapes, dimensions, and force sensitivities. Scanning electron microscope (SEM) images of silicon microcantilevers used as AFM probes with different geometry and sizes: (a) commercial rectangular cantilever with integrated tip (Nanosensors GmbH & Co.); (b) commercial triangular (in order to minimize torsional deflections) cantilever with integrated tip (Digital Instruments); (c) interdigitated cantilever proposed by Manalis et al.[42]. (Reproduced with permission of R. Berger from[82].)

available with different shapes, dimensions, and force sensitivities (Fig. 1). Measurements in the 10^{-11} N range – at the level of single bio-molecular pairs – are possible[4-10].

During the last decade SPM has been applied in other scenarios where the properties of specific micro-cantilevers are used to detect changes in temperature, surface stress, mass, and magnetization in nano-gram amounts of materials. Signals from such small quantities are often not accessible by means of macroscopic techniques or require complex analytical tools. The use of microcantilever sensor techniques is inexpensive compared with other macroscopic analytical tools and can be operated in every laboratory.

Transduction principles

A cantilever composed of a sandwich of materials with different thermal expansion coefficients bends as a function of ambient temperature (Fig. 2b). This 'bimetallic' transduction principle allows the measurement of changes in temperature[11] as small as 10^{-5} K. Such sensors have been employed for photothermal measurements by using a specific light absorband[12-15] or as micro-calorimeters to study the heat evolution in chemical reactions at a catalytic layer situated on top of the sensor[11]. Enthalpy changes at phase transitions of only 500 pJ in pico-gram quantities of material attached to the sensor apex have been analyzed reliably[16,17]. Bimetallic microcantilevers can perform photothermal spectroscopy of thin overcoats[18] with 150 fJ sensitivity and sub-millisecond time resolution[17]. Theoretical estimates show these sensors can detect heat changes with atto-Joule sensitivity[11,19].

Microcantilevers can also be operated as precise balances (Fig. 2c) by measuring their vibrational characteristics (oscillating mode). Additional mass loading at the apex of a cantilever sensor decreases its resonance frequency (Eq. 1). Changes in viscosity or density of the environment also influence the vibrational characteristics of the sensor. Such a viscosimeter operation principle is shown in Fig. 2d and has been proposed by several researchers[20-22]. When operated in the oscillating mode it is important to know that during adsorption or desorption processes the deposited material can change cantilever mechanical properties, e.g. stiffness.

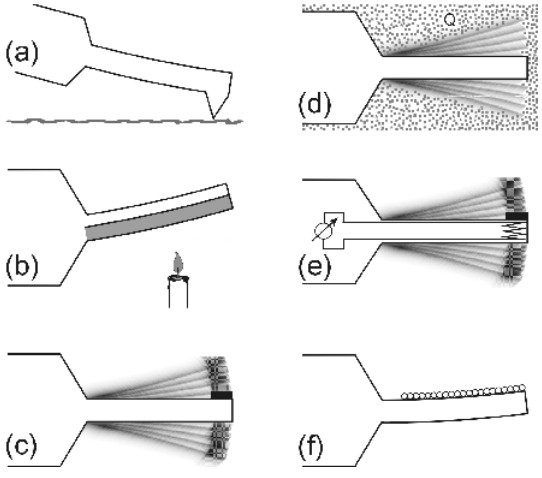


Fig. 2 Schematic drawings (side view) of a variety of possible cantilever transducer principles: (a) force sensor with integrated tip for AFM; (b) "bimetallic" temperature and heat sensor; (c) mass loading sensor; (d) medium viscoelasticity sensor; (e) thermogravimetric sensor; and (f) stress sensor.

Water adsorption on a gelatin coated cantilever causes an increase in resonance frequency[23-25], in contradiction to the effect of the mass increase (Eq. 1). This crosstalk between mass and stiffness changes can be separated if the sensing layer is concentrated at the free end of the cantilever (Fig. 2c). In this case the shift in resonance frequency can be directly related to the change in mass by the following equation:

$$\Delta m = K/4\pi^2\{1/f_1^2 - 1/f_0^2\} \qquad (1)$$

where K is the cantilever spring constant, f_0 and f_1 are the resonance frequencies before and after adsorption. By only allowing adsorption at the apex , however, the adsorption area is reduced and consequently the sensitivity. By using a porous material, e.g. a zeolite, as a 'sensing sponge' one can enhance the sensitivity[26]. Berger *et al.*[27] investigated thermogravimetric analysis using an oscillating, heated piezoresistive cantilever in helium gas (Fig. 2e)[28]. Theoretical estimates based on commercially available cantilevers show a minimum detectable mass density of 0.67 ng/cm^{-2}, comparable to acoustic sensors like surface acoustic wave oscillators (SAW) and quartz crystal microbalances (QCM). Where the active area of the structure is taken into account, a minimum detectable mass of 10^{-15} g has been achieved[29].

Unfortunately, when a sensor is operated in a liquid in oscillating mode, both the resonance peak and its quality factor, Q, shift toward lower values because of damping[30]. This considerably reduces the achievable resolution in terms of minimum detectable mass change. Mehta *et al.*[31] and Tamayo *et al.*[32] propose methods to enhance the Q factor of oscillating cantilevers in liquids and, hence, the resolution.

In liquids – the natural environment for biochemical reactions – a bending which results in a static deflection, even if only a few nanometers, is easily detectable. Cantilevers are, therefore, often operated in this mode as surface stress sensors (Fig. 2f). It is well known that a uniform surface stress acting on an isotropic material tends either to increase (compressive stress) or decrease (tensile stress) the surface area. If this effect is not compensated by an equal stress on the opposite side of a thin plate or beam, it will permanently bend the whole structure (Fig. 3). Many years ago Stoney[33] measured deposition-induced bending of beams in a electrochemical environment and related the differential surface stress change between the opposite faces of a thin beam, with the resulting radius of curvature. By measuring the deflection, the difference in surface stress between the two sides can be calculated. Adsorption of molecules to a surface causes a change in the surface stress.†

Ibach studied surface stress evolution on crystalline cantilevers induced by adsorption of single atoms both experimentally[37,38] and with finite element analysis[39]. When dealing with complex molecules like proteins, however, there are several other possible sources of stresses. Electrostatic interaction between neighboring adsorbates, changes in surface hydrophobicity, and conformational changes of the adsorbed molecules can all induce stresses which may overlay each other and make changes not directly related to the receptor-ligand binding energy or the rupture force. As an example of the complexity of the issue, Wu *et al.*[40] recently observed how adsorption of complementary single-stranded (ss) DNA onto the cantilever surface can induce either compressive or tensile stress depending on the ionic strength of the buffer in which the hybridization takes place. They interpret this behavior as the interplay between two opposite driving forces: a reduction of the configurational entropy of

† Surface stress and tension are related but distinct quantities for solid surfaces. For a detailed discussion about how surface stress and free energy are related see[34-36].

What is a biosensor?

A biosensor, as any other sensing device, can be divided into three main components: a ***detector*** which recognizes the signal of interest, a ***transducer*** which converts the signal into a more useful output, typically an electronic signal, and a ***read out system*** which filters, amplifies, displays, records, or transmits the transduced signal. A biosensor employs a biological or biochemical ***detector***, which can range from single proteins and enzymes up to whole cells and microorganisms.

Biosensors can be classified by: the detector type, e.g. immunosensors or enzymatic sensors; the transduction principle, e.g. amperometric, piezoelectric or (micro)mechanical; and the application, e.g. clinical sensors or environmental sensors.

In the case of cantilever biosensors the cantilever transduces the recognition event from its receptor-coated surface into a mechanical response which can be detected using different methods. MT

the adsorbed DNA after hybridization which tends to lower the compressive stress, while the intermolecular electrostatic repulsion between adsorbed DNA increases the stress.

Cantilever deflection detection

With the introduction of AFM, several deflection detection methods can measure microcantilever deflection with sub-Angstrom resolution. Optical and electrical methods can be implemented in cantilever based (bio)sensors as well.

The most well used AFM method is the 'beam bounce' or optical lever technique. Visible light from a low power laser diode is focused on the free apex of the cantilever, which acts as a mirror. Commercial AFM cantilevers can be coated with a thin layer of gold to increase reflectivity. The reflected beam hits a position sensitive or split photodetector (Fig. 4). When the cantilever bends, the reflected laser light moves on the photodetector surface and the distance travelled is proportional to the cantilever deflection.

Another optical deflection detection method is based on the interference between a reference laser beam and the reflection from the cantilever. Interferometry is highly sensitive and provides a direct and absolute measurement of the displacement. However, it only works well for small displacements (absolute deflection is defined only within a single wavelength) and is technically demanding (light has to be brought close to the cantilever to get a reflection). For this purpose, Rugar *et al.*[41] position the cleaved end of an optical fiber a few microns from the free end of a cantilever.

An alternative approach uses interdigitated cantilevers as an optical diffraction grating. The reflected laser light forms a diffraction pattern, where the intensity is proportional to cantilever deflection. This method is already implemented for AFM[42,43], as a physical sensor in accelerometers[44], and for infrared imaging[45]. It is proposed for chemical sensing too[46].

Capacitive sensors measure displacement as a change in the capacitance of a plane capacitor. Blanc *et al.*[47] reports microfabricated capacitive sensors for AFM where the cantilever is one of the capacitor plates. This technique is highly sensitive and can provide absolute displacement, but it is not suited to measure large displacements and does not work in electrolyte solutions because of the faradaic currents between the capacitor plates. It is therefore of limited use in biosensing applications.

A more interesting method uses piezoresistive cantilevers. When a piezoresistive material like doped silicon is strained,

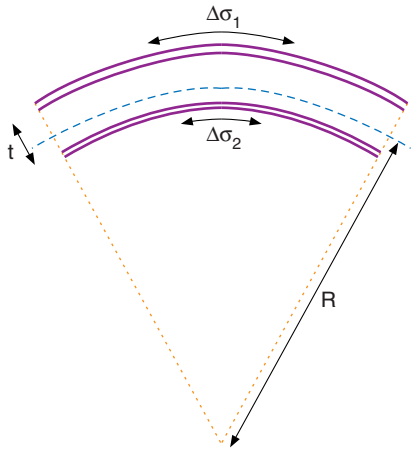


Fig. 3 Lateral view of a thin beam of thickness t subjected to compressive surface stress changes $\Delta\sigma_1$ and $\Delta\sigma_2$. The beam bends around a neutral plane with a constant radius of curvature R.

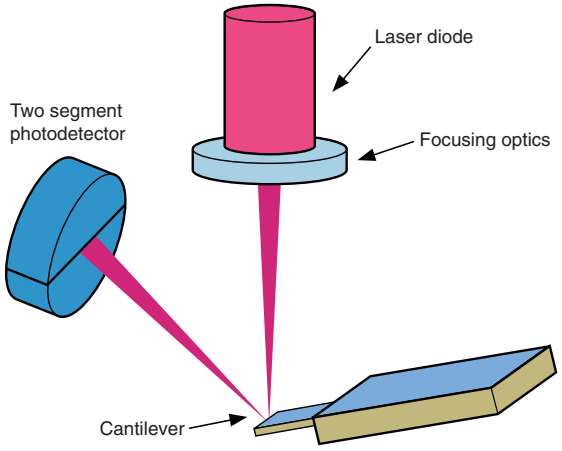


Fig. 4 Optical lever deflection detection method.

it changes its electrical conductivity. Piezoresistive sensors are therefore ideally suited to monitor stresses. Such stress sensors can be integrated on a cantilevered structure with resistivity measurable with a simple Wheatstone bridge[48,49]. Recent improvements allow the fabrication of thin and passivated resistors on cantilevers[50,51] that can be used in electrolyte solutions by avoiding faradaic currents. To compensate thermal drifts, a symmetrical configuration has been developed where the output signal is a differential deflection between sensing and reference cantilevers[50,51].

Piezoresistive cantilevers have some advantages compared with standard optical techniques: no optical components or laser alignment are needed; read-out electronics can be integrated on the same chip using CMOS fabrication; they are unaffected by optical artifacts arising from changes in the

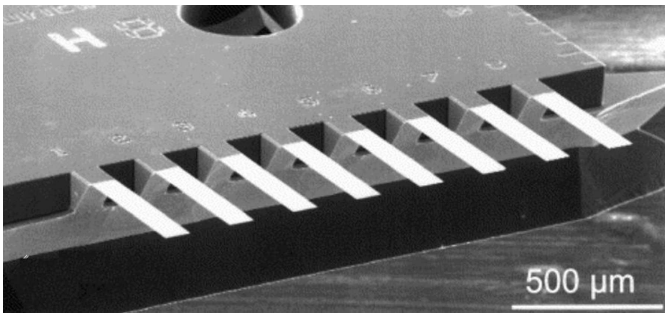
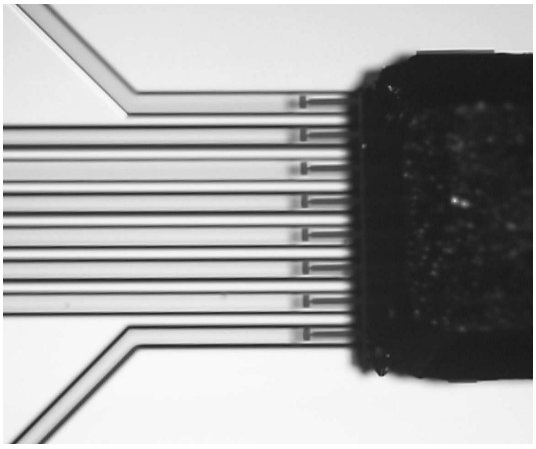


Fig. 5 (a) SEM image of an array of eight silicon microcantilevers fabricated at the Micro- and Nano-mechanics Group, IBM Zurich Research Laboratories. Each cantilever is 1 µm thick, 500 µm long, and 100 µm wide, with a pitch of 250 µm, spring constant 0.02 N/m. (Reproduced with permission from[78].)



(b) Optical micrograph of the IBM array above a microfluidic channel network with the same 250 µm pitch. (Reproduced with permission of R. Berger from[82].)

optical properties of the medium surrounding the cantilever (e.g. a change in the refracting index when exchanging two different solutions); and work in non-transparent solutions.

Piezoresistive cantilevers can also vary their surface temperature by increasing the electrical current flow through the resistor layer. This could be implemented as a tool for breaking the ligand-receptor binding, thus regenerating the sensing layer in biosensing applications.

Cantilever surface functionalization

The receptor layer deposited on a cantilever surface directly affects sensor selectivity, reproducibility, and resolution. One wants to deposit a thin (to avoid changes in mechanical properties of the cantilever), uniform (to generate a uniform stress), and compact (to avoid interactions with the solid substrate beneath) layer of receptor molecules. It should be stable and robust, with the receptors covalently anchored to the surface while retaining enough freedom to interact with their specific ligand. Receptor activity should be maintained over time and withstand regeneration of the sensing layer, if the sensor has to be reused several times. Most of these requirements are common to other biosensors and, in fact, the proposed coating techniques and procedures are shared with other transducing principles. Noble metals are often deposited either as a substrate to anchor successive layers or as catalyst for gas adsorption. Evaporation and sputtering allow precise control of the layer thickness and distribution.

An easy and popular method to create ordered monolayers uses self-assembling monolayers (SAMS)‡, such as alkane chain molecules with thiol groups on gold substrates[53,54] or silanes on silicon substrates[55,56]. SAMS spontaneously form uniform, densely packed, robust (covalent binding) monolayers, which can be synthesized with different chain lengths and end groups with specific chemical properties. They are therefore ideally suited to act as cross-linkers to anchor the receptor molecules to the substrate.

To form a thiol monolayer on one side of a cantilever, gold has to be evaporated and the whole cantilever incubated in the thiol solution or exposed to thiol vapors. Berger *et al.*[57] report detecting surface stress changes during the formation of alkanethiol monolayers on gold coated cantilevers. Rinsing the cantilever removes the unspecifically adsorbed thiol on the opposite side. Raiteri *et al.*[58] have developed a simple multi-step procedure which allows the coating of each cantilever side with different thiol monolayers.

Another way to add specific functional groups to a surface is to attach (graft) polymers of an appropriate structure. To enhance polymer deposition on surfaces with only a small number of surface reactive sites, plasma treatment can be used[59]. Betts *et al.*[60] deposit thin (150 nm) films of different polymers by spin coating, using a focused ion beam mill to remove unwanted polymeric coating from the opposite side.

Organic layers can be created on solid substrates without the need of reactive surface sites. Langmuir-Blodgett (LB) deposition transfers ordered layers of amphiphilic molecules from the water/air to the solid/air interface and allows precise control of multilayer formation[61]. It is, however, quite tricky to avoid the deposition on both cantilever sides. Sol-gels can be used for surface functionalization too. They allow the creation of layers of porous materials with controlled pore size. Increased active area can act as a catalyst with the pores serving as a mechanical filter to improve the specificity

‡ For a complete review on the structure and growth of SAMs see[52].

of the interaction[62]. Sol-gel spin-coated cantilevers can distinguish between vapor phase analytes of varying chemical composition, as well as varying concentration of an analyte[63].

It is also crucial to characterize the deposited layers and check the specific activity of the receptors once anchored on the cantilever surface. This can be done in different ways, depending on the nature of the receptors. Immuno-based sensors could use enzyme linked immunosorbent assay (ELISA) to monitor the amount of active antibodies quantitatively, fluorescence microscopy to determine spatial distribution, and AFM for morphology[64].

Biosensing applications

The sensitivity and versatility of microcantilevers for physical and chemical sensing has recently attracted interest for biosensing applications too. When compared to ELISA, a standard assay for detecting protein markers, cantilever sensors could be faster and cheaper since there is no need to attach fluorescent tags to molecules for detection and parallel operation is possible (each cantilever coated with a different marker). It is also possible to perform continuous real-time monitoring of receptor-ligand interactions.

Baselt *et al.*[65] propose using microfabricated cantilevers as force transducers to detect the presence of receptor-coated magnetic beads, which stick onto the functionalized cantilever surface. In principle it is possible to detect the presence of a single micrometer size magnetic bead stuck to a cantilever by applying an external magnetic field and measuring the deflection. Coating the cantilever surface with specific receptors and labelling the analyte with the magnetic beads could yield an extremely sensitive sensor.

Antonik *et al.*[66] propose sensing the mechanical responses of living cells, cultured directly onto the cantilever surface, to external chemical stimuli. With a microcantilever operated in the oscillating mode, Ilic *et al.* [67] can weigh and count, in air, the number of bacteria adsorbed onto an antibody-coated cantilever by monitoring shifts in its resonance frequency.

With cantilevers operated in static stress mode, Butt[68] can follow unspecific bovine serum albumine (BSA) adsorption on a hydrophobic microcantilever surface. Raiteri *et al.*[69] show that the specific binding of a herbicide to its antibody-coated cantilever causes a permanent deflection. Moulin *et al.*[70] can differentiate between the adsorption of low density lipoproteins and the oxidized form on heparin and can monitor the surface stress induced by slow conformational changes of proteins like BSA adsorbed onto a gold surface[71]. Wu *et al.*[72] report a cantilever-based biosensor that is sensitive enough to be a diagnostic assay for the protein markers of prostate cancer – a concrete alternative to ELISA.

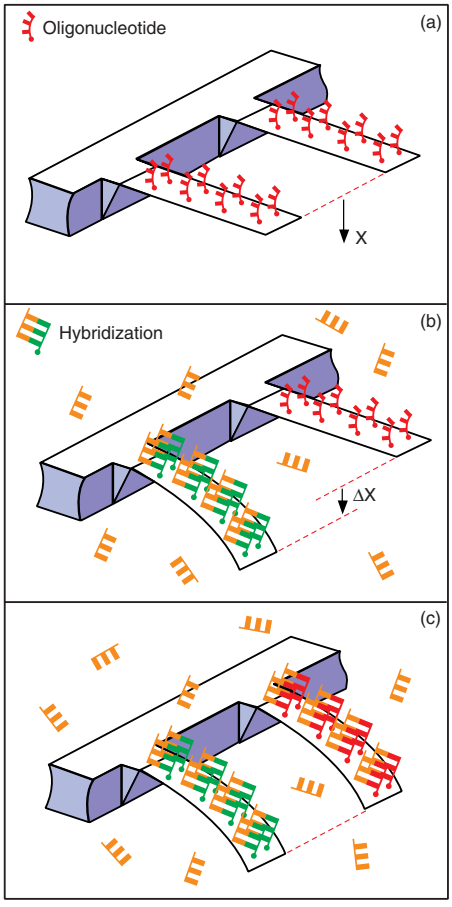


Fig. 6 Scheme of the hybridization experiment done at IBM Zurich. Each cantilever is functionalized on one side with a different 12mer oligonucleotide (red or blue). Oligonucleotide base sequences differ by one base only. They are synthesized with a spacer and a thiol group at one end to covalently bind to the gold coated cantilever surface: (a) differential deflection signal is set to zero; (b) after injection of the first complementary oligonucleotide (green), hybridization occurs on the cantilever providing the matching sequence (red), increasing the differential signal $\Delta x = 9$ nm; (c) injection of the second complementary oligonucleotide (yellow) causes the cantilever functionalized with the second oligonucleotide (blue) to bend. (Reprinted with permission from[73]. Copyright 2000 American Association for the Advancement of Science.)

Cantilever sensors also show great potential in genomics research. One hot topic is the detection of single base-pair variations in DNA (single nucleotide polymorphisms, SNPs), a source of biological diversity and several diseases. Fritz *et al.*[73] monitor ssDNA hybridization with two microcantilevers in parallel where their differential deflection allows discrimination of two identical 12mer oligonucleotides with a single base mismatch (Fig. 6). Hansen *et al.*[74] obtain a similar result using 10mer oligonucletides.

Moving to market

Microcantilever-based sensors have now started attracting interest at the industrial/market level.

One of the first and most active research groups dealing with cantilever-based sensors is at Oak Ridge National Laboratories. In 1998 their cantilever technology was licensed to Graviton, Inc. (www.graviton.com) – a startup aiming to integrate cantilever-based sensors with MEMS and wireless technology to create networks of remotely operated physical and chemical sensors for industrial, home and consumer uses.

In March 2000 another US-based startup company, Protiveris, Inc. launched (www.protiveris.com), with the objective of bringing a microcantilever-based biosensor to market for proteomic drug discovery applications.

Cantion A/S was founded in late 2001 by researchers at the Danish Microelectronic Center to commercialize chips with piezoresistive cantilevers for biochemical uses (www.cantion.com). MT

Cantilever arrays and the future

In biomedicine and biotechnology only small amounts of reagents are usually available. Hence high sensitivity and screening throughput are needed. In addition, reference measurements are required to improve signal quality.

The next breakthroughs will therefore be the development of techniques capable of detecting, in a simple way, the deflection of large arrays of cantilevers (>100) in liquids; and reproducible and reliable ways to coat cantilever surfaces with robust layers of bioreceptors. The latter is a common problem and the option of averaging over large numbers of cantilevers identically coated could improve the signal to noise ratio. Technical issues do arise with arrays of tiny cantilevers (Fig. 5a). One solution is to use a multi-channel fluidic system (Fig. 5b), where each cantilever is immersed in a channel filled with a chemical reagent. Another approach is to spray small amounts of the coating solution in the proximity of each cantilever using ink-jet printer technology.

Researchers at IBM in Switzerland are investigating an array of eight cantilevers (Fig. 5a), using a multiplexed readout system based on the optical lever technique[75]. They propose the array as an artificial nose to detect vapors in air by coating the cantilever with different polymer layers and monitoring both static deflection and resonance frequency changes[76-78]. For larger arrays, piezoresistive cantilevers and optical interference pattern based techniques look promising.

We have seen how cantilever based sensors are extremely versatile: they can be operated in air, vacuum, or liquid environments; and can transduce a number of different signals, such as magnetic, stress, electric, thermal, chemical, mass, and flow, into a mechanical response detectable by various methods. They are already populating laboratories worldwide as force sensors in AFM set-ups and are close to industrial exploitation as physical sensors. Sensors are also the focus of attention in biomedicine and biotechnology where they offer advantages in sensitivity, time response, analysis time, fabrication cost, miniaturization, potential for large sensor arrays, and integration with MEMS technology. Arrays of 1024 cantilever elements are also being explored as ultra-high density storage devices[79,80].

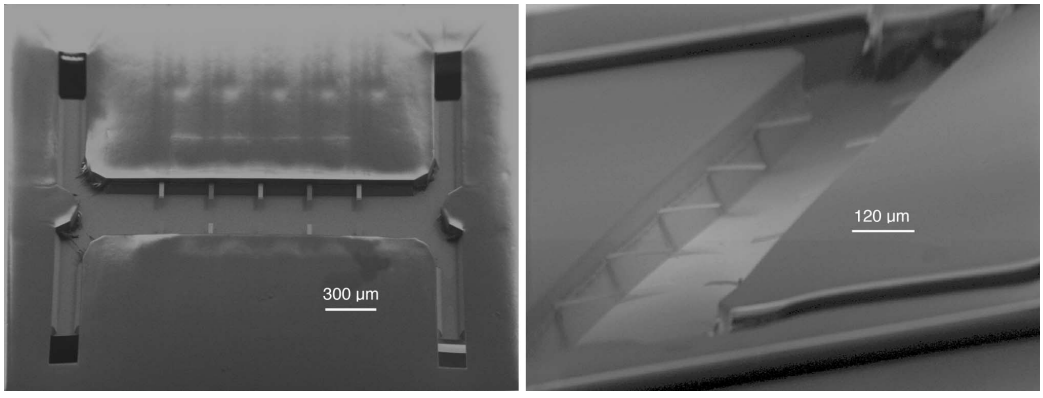


Fig. 7 SEM micrographs of the sensor chip developed by Anja Boisen's group at the Mikroelektronik Centret, Technical University of Denmark. The left image shows a top view of the whole channel (400 µm wide, 3 µm long for a total volume of 0.14 µl), with 10 piezoresistive cantilevers and 4 inlets/outlets. The right image shows a side-view of a part of the channel where the thin cantilevers (125 µm long, 40 µm wide, and 520 nm thick) placed in the middle of the sidewall can be seen. (Reproduced with permission.)

Further developments should bring full integration with microfluidic handling systems, other analytical techniques, and signal extraction electronics. Towards this end, Fig. 7 shows a cantilever sensor array with a piezoresistive readout and microfluidic handling system integrated onto a silicon chip[81]. Technological improvements will allow fully encapsulated piezoresistive layers, which can be operated in electrolyte solutions. We foresee that MEMS will provide intelligent ways for biologists to create a 'handling and analysis laboratory on a single chip'.MT

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