

TUTORIAL

Nanomechanics from atomic resolution to molecular recognition based on atomic force microscopy technology

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Abstract

Atomic force microscopy (AFM) is a technique to image surfaces with unprecedented vertical and lateral resolution. Many related techniques have been derived from AFM, taking advantage of local interactions between a tip on a cantilever and a surface. However, cantilevers can also be used for sensing applications. These so-called nanosensors feature extreme sensitivity for the detection of chemical vapours or adsorption of molecules. Upon adsorption to the cantilever surface, the molecules cause the cantilever to bend. Thus physical, chemical or biochemical processes are directly transduced into nanomechanical motion. We show that measurement of the deflection of a single cantilever might be misleading. Reliable information can only be obtained by using a sensor cantilever and at least one reference cantilever integrated into an array. We have built an electronic nose using polymer layers as partially selective cantilever coatings to recognize chemical vapours and odours by evaluating the cantilevers' bending pattern. Major applications lie in the fields of process and quality control, biosensing, medical diagnostics, molecular recognition and proteomics.

1. Introduction

Technology today is an essential part of our economic, physical and societal environment and its importance will continue to grow. Within the past 30 years, the transformation of scientific knowledge into commercial products has reached a pace unimaginable in the 1960s. A prominent example illustrating this transfer of basic scientific knowledge is the laser. Invented in 1958, it provides numerous applications today in communication technologies, the entertainment industry, medicine, engineering of materials and many more. In addition to these developments there is an urgent need to advance the basic science involved, and in particular to provide the required interdisciplinary scientific culture.

At the beginning of a new millennium, three major scientific disciplines were considered to have an unprecedented potential for industrial applications. These are the fields of in-

formation science and life sciences, which in turn are increasingly influenced by nanoscience. The fundamental research in the overlapping areas requires an interdisciplinary approach and inevitably will lead to a new industrial revolution. The underlying science involves the basic building blocks and length scales of matter in biology, chemistry, physics and engineering.

Nature is the best example of a system that functions on the nanometre scale and where the materials, energy consumption and data handling processes are optimized. Integration of physics, chemistry and the life sciences into the field of nanoscience and nanotechnology is a novel concept that will generate new synergies in research and education. This promises to create a new generation of scientists able to advance nanoscale science on a broader basis of knowledge and experience. Nanotechnology is expected internationally to become a driving force of the world economies within the next 10–20 years (see figure 1).

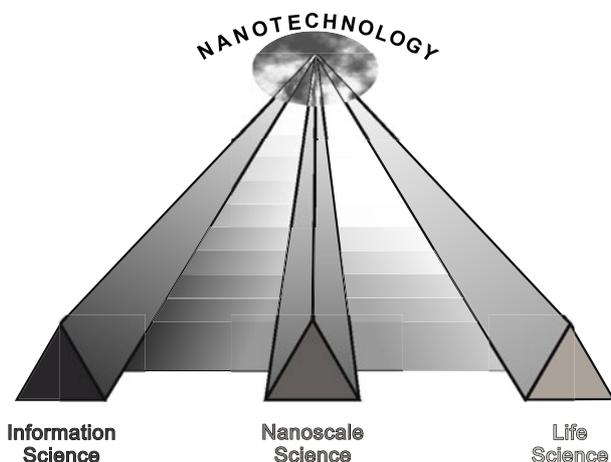


Figure 1. The individual disciplines of physics, chemistry, biology, engineering and information science will merge into a highly interdisciplinary environment of nanoscale science, life sciences and information sciences to form the common field of nanotechnology.

2. From atomic resolution to molecular recognition

Since its invention in 1986 [1], the atomic force microscope (AFM) has proved its suitability in various fields of application. First designed as an instrument to image the surfaces of nonconductive materials with high lateral and vertical resolution, the technique has been adapted for various environments, such as vacuum, fluidics, ambient, low temperatures and magnetic fields, as well as for chemistry and biology applications. The capability to investigate surfaces with unprecedented resolution using the techniques of scanning probe microscopies introduced a wealth of related techniques using probes with local interaction (see figure 2). The interaction force may be the interatomic forces between the atoms of the AFM tip and those of a surface, short-range van der Waals forces or long-range capillary forces, or stick-slip processes producing friction forces. Modifying the AFM tip chemically allows various properties of the sample surface to be measured. The AFM tip can be driven in an oscillating mode to probe the elastic properties of a surface (elastic modulus spectroscopy). Local charges on the tip or surface lead to electrostatic forces between tip and sample, which allow a sample surface to be mapped, i.e. local differences in the distribution of electric charge on a surface (electrostatic force microscopy) to be visualized. In a similar way magnetic forces can be imaged if the tip is coated with a magnetic material, e.g. iron, that has been magnetized along the tip axis (magnetic force microscopy). The tip probes the stray field of the sample and allows the magnetic structure of the sample to be determined. A strong dependence of the resolution on the tip-sample distance is observed. Information on force gradients can be obtained by cantilever oscillation techniques. At high oscillation frequencies (cantilevers with high resonance frequency), further information on interatomic forces between tip and sample can be obtained. Depending on the oscillation amplitude we use the terms ‘tapping mode’ or ‘dynamic force microscopy’. Dynamic force microscopy is able to provide true atomic resolution on various surfaces under ultrahigh vacuum conditions and allows force spectroscopy on specific sites. Material properties can be locally discerned

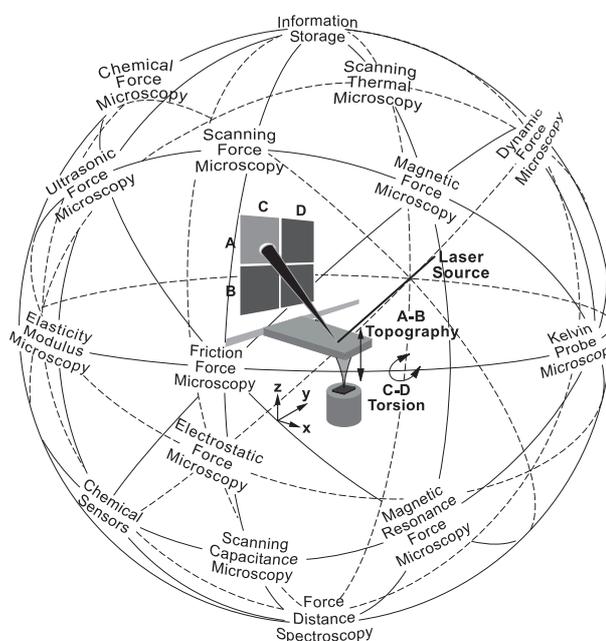


Figure 2. The universe of AFM-based microscopy and local sensor techniques.

using ultrasonic force microscopy. Various other quantities can be measured if the tip is functionalized as a local measurement tool, e.g. as a very small thermocouple to measure temperature differences in scanning thermal microscopy. Locally resolved measurement of the chemical potential is the goal of Kelvin probe microscopy, whereas the capacity change between tip and sample is evaluated in scanning capacitance microscopy.

In addition to imaging surfaces, AFM can also be used to modify surfaces and perform molecular manipulation down to the level of individual molecules or atoms. By depositing, removing or modifying material from the tip and/or sample surface, a surface can be modified locally for the storage, retrieval and erasing of information. The operation mode of acquiring force distance curves (measurement of forces as a function of tip-sample separation) allows us to draw conclusions regarding the material characteristics of surfaces and their chemical properties. With bonds established between the tip of a scanning force microscope and a molecule tethered to a surface, force can be exerted very locally on a single molecule and thus the strength of bonds and the forces required to break individual bonds can be investigated (force distance spectroscopy and single-molecule spectroscopy). The mechanical detection of electron or nuclear magnetic resonance (magnetic force resonance force microscopy, MRFM) has shown improved sensitivity compared to induction-based techniques. MRFM might finally lead to a way of locally mapping a surface with chemical resolution. The development of a technique that might be able to provide a chemically resolved image of a sample surface (chemical force microscopy) has revealed that the cantilever itself is a very sensitive tool for observing chemical reactions and processes.

We used local probes such as cantilevers—microfabricated beams of silicon, for example—to perform local experiments using the surface of a cantilever. If the surface of a cantilever

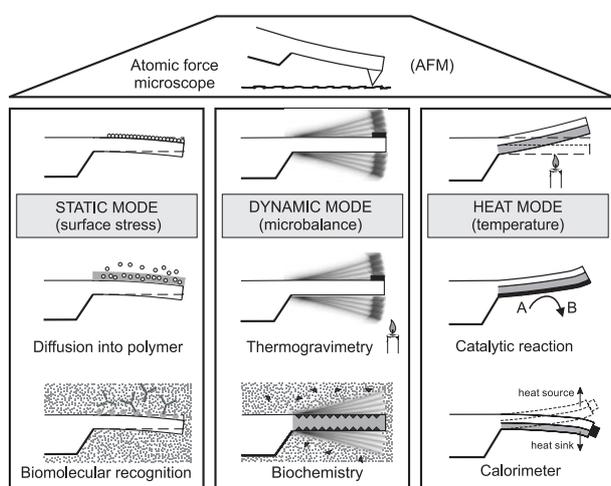


Figure 3. Cantilever sensor operation in the static mode (left), the dynamic mode (middle) and the heat mode (right).

is functionalized in such a way that a chemically active and a chemically inactive surface is obtained, chemical or physical processes on the active cantilever surface can be observed using the temporal evolution of the cantilever's response. Cantilevers can be used as a nanomechanical sensor device for detecting chemical interactions between binding partners on the cantilever surface and in its environment. Such interactions might be produced by electrostatic or intermolecular forces. At the interface between an active cantilever surface and the surrounding medium, the formation of induced stress, the production of heat or a change in mass can be detected. In general, one of the binding partners is placed on a cantilever, while the other binding partners are present in the environment. Figure 3 shows the variety of detection modes using such cantilever sensors. We distinguish three main strands of sensor types: static mode, dynamic mode and heat mode.

In the *static mode* the static bending of the cantilever beam due to external influences and chemical/physical reactions on one of the cantilever's surfaces is investigated (see left column of figure 3). The asymmetric coating with a reactive layer on one surface of the cantilever favours preferential adsorption of molecules on this surface. In most cases, the intermolecular forces in the adsorbed molecule layer produce a compressive stress, i.e. the cantilever bends down if its reactive surface is its upper one. If the reactive layer is a thin layer of polymer, molecules from the environment will diffuse into the polymer layer and cause it to swell, resulting also in a downward bending of the cantilever. Finally, in liquid or biochemical environments, we can observe a downward bending of the reactive cantilever surface when molecular recognition reactions are taking place on the functionalized surface of the cantilever. Processes such as electrostatic repulsion between adsorbed biomolecules or steric effects due to the fact that the adsorbed molecules require additional space on the surface are responsible for the formation of compressive stress on cantilevers in fluids. Further examples of surface stress-related cantilever experiments can be found in the literature [2–5].

In *dynamic mode* the cantilever is driven at its resonance frequency (see the middle column of figure 3). If the mass of the oscillating cantilever changes owing to additional mass

deposited on the cantilever, or if mass is removed from the cantilever, its resonance frequency changes. Using electronics designed to track the resonance frequency of the oscillating cantilever, the mass changes on the cantilever are derived from shifts in resonance frequency. The cantilever can be regarded as a tiny microbalance, capable of measuring mass changes of less than 1 pg [6]. The oscillating cantilever set-up can be operated in various environments, for example in a small chamber that can be externally heated. If a small amount of sample material is attached to the apex of the cantilever, we can investigate the response of that piece of material as a function of the thermal programme imposed. We have shown [7] that the dehydration reaction of copper sulfate pentahydrate into water-free copper sulfate upon heating can be investigated on a sample mass of a few micrograms (thermogravimetry), whereas in classical thermogravimetry milligram quantities are required. If a biochemically active layer is deposited onto the surface of a cantilever, the mass change during molecular recognition reactions can be observed directly using the oscillating cantilever technique. Two comments should be added here. First, the coating with the active sensor layer can be applied on both surfaces of the cantilever to increase the active surface where the mass change takes place. Second, oscillating a cantilever in liquids results in a very low quality factor, which complicates the exact determination of the resonance frequency of the cantilever. However, the quality factor may be enhanced electronically. Examples of measurements in the dynamic mode can be found in the literature [6, 7].

In the *heat mode* the cantilever is coated asymmetrically on one surface with a layer having different thermal expansion properties than those of the cantilever material. Suitable materials for coating silicon cantilevers are, for example, aluminium or gold. If such a cantilever is subjected to external temperature changes it will bend due to the differing thermal expansion coefficients of the cantilever material and the coating layer. Deflections corresponding to temperature changes in the microkelvin range can be measured easily. The coating layer can also be catalytically active, e.g. a platinum layer facilitates the reaction of hydrogen and oxygen to form water. In this case, heat is directly generated on the surface of the cantilever, which causes the cantilever to bend owing to the differing thermal expansion coefficients of silicon and platinum. The cantilever surface can also be used as a platform to perform thermal experiments. A small amount of a sample (typically a few hundred nanograms) can be attached directly to the apex of a cantilever and be subjected to a thermal profile. We studied phase transitions of an alkane sample of only a few nanograms in mass using the cantilever technique and found enthalpy changes that compare very well with data measured on milligram quantities of material. Thus the cantilever sensor technique is a suitable method if only very small amounts of material are available [8, 9]. A way to obtain chemical information on adsorbing molecules has been achieved using cantilevers by a method known as photothermal spectroscopy [10]. By integration of local heating elements and piezoelectric deflection detection, the cantilever concept can be modified for storage applications using thermomechanical writing and erasing schemes. Recently, the technique has been scaled up to more than 1000 cantilevers ('millipede' storage device) [11].

In summary, we have seen that cantilevers can be used not only as local probes for imaging a surface, but also as tools to measure physical, chemical or biochemical properties of materials. In the next section we present a method for extracting reliable information from multiple cantilever responses.

3. Extracting information from cantilever responses

Cantilevers might bend owing to a variety of reasons, such as forces due to the adsorption of molecules, the accumulation of interfacial or surface stress, surface tension, an increase of mass loading, momentum transfer, forces due to electrostatic charges or magnetic fields, thermal effects, reaction heat, configurational changes on the surface, rearrangement of atoms and molecules on the surface, and many more. The art of taking measurements with cantilevers is to distinguish between the contribution of desired effects to the cantilever response and the influence of undesired effects. As it is not easy to judge from the response of a single cantilever whether the observed deflection results actually from a physical, chemical or biochemical process or from an undesired artefact, we have to achieve close control over the properties of the cantilever, for example (a) its mechanical properties such as the accuracy of its length, width and thickness, (b) the occurrence of mechanical defects due to the microfabrication process, (c) how it is attached to the chip support, (d) the chemical homogeneity of its surfaces and (e) the reactivity of its surface to other gases, vapours or liquids, to name just a few. A convenient way to test the reproducibility of cantilever fabrication is to compare the properties of many cantilevers. Test criteria might include the spring constant of the cantilever, its resonance frequency, the quality factor of the resonance or the bending behaviour upon exposure to temperature changes or chemical contamination. In addition to such geometrical and chemical differences between individual cantilevers, there is the requirement to perform an experiment several times in a very reproducible way. If we want to observe a certain reaction using a chemically functionalized cantilever under well-defined conditions (e.g. temperature, pressure, chemical environment, gas or liquid flow rates), we shall have to be able to establish exactly the same conditions in all the experiments. To study the affinity of target molecules to probe molecules of the cantilever coating, the experiment should be repeatable under identical conditions to exclude unwanted artefacts. To facilitate this procedure, we use more than one cantilever at the same time in our experiments. A cantilever with an inert coating, i.e. one that does not take part in the reactions, can be used as a reference cantilever for the reaction to be observed.

To overcome possible problems regarding the reproducibility of cantilever microfabrication or to establish identical conditions in consecutive experiments, it is convenient to integrate a reference cantilever together with the chemically active (sensor) cantilever on the same chip. It is extremely important that the microfabrication process yields high precision structures in such a way that the reproducibility in resonance frequencies is better than a few tenths of a per cent. Thus the experiment can actually be performed under identical conditions for both the chemically modified sensor cantilever and the ideally inert reference cantilever. By numerical calculation of

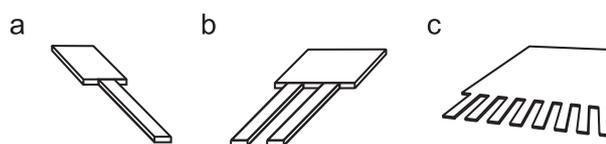


Figure 4. (a) Single cantilever. No thermal drift compensation is possible. For reproducible results the upper and lower surface of the cantilever should be chemically well defined. If the upper surface is chemically active, the lower surface should be passivated; otherwise the signal will be convoluted with undesired effects. (b) Dual cantilever. One cantilever is coated on its upper surface with a chemically active layer (sensor cantilever), whereas the other is inactive (reference cantilever). If the lower surface of both cantilevers is reactive in the same way, this effect can be compensated by numerical calculation of the difference in responses (differential deflection measurements). Thermal drift occurring on both cantilevers will be cancelled out by differential deflection measurements. (c) Cantilever array. Several cantilevers can serve as sensor or reference cantilevers, allowing various differential deflection signals including thermal drift compensation.

the difference between the responses of the sensor and those of the reference cantilever, the true reaction response can be extracted from the data. In an experimental set-up with a sensor cantilever and a reference cantilever the minimal requirements of sensing reactions using cantilevers are met (see figure 4). Adsorption processes on the nonfunctionalized surface of both cantilevers are cancelled out in the calculation of the difference in responses, as are thermal drifts affecting both cantilevers to the same extent. Using many cantilevers (e.g. an array of eight cantilevers) opens up the possibility of exposing several sensor cantilevers and reference cantilevers in a single experiment under identical conditions in terms of gas or liquid flow rates, temperature and pressure conditions. The use of cantilever arrays renders much more reliable results and is more efficient because several experiments can be performed at the same time. In the next section we will see that it is mandatory to evaluate numerically the difference in responses of the active sensor cantilever and the reference cantilever. In this so-called differential deflection response, undesired artefacts influencing both cantilevers to the same extent are cancelled out.

4. Example of differential deflection measurements: DNA hybridization

This example is to demonstrate that differential deflection measurements are essential to obtain relevant information on a physical, chemical or biochemical process. It will demonstrate very clearly that the measurement of the deflection of only a single cantilever will yield a misleading result that might give rise to an incorrect interpretation of the cantilever deflection curve. The biochemical system discussed here involves a DNA hybridization experiment using a thiolated 12-mer oligonucleotide sequence from the Bio B biotin synthase gene (EMBL accession number: J04423). We call this surface bound probe Bio B1. Its base sequence is 5'-SH-C₆-ACA TTG TCG CAA-3' (C₆ is a spacer). The Bio B1 probes are immobilized by thiol binding on the gold-coated upper surface of a cantilever in an array. Its target complement is Bio B1C (5'-TTG CGA CAA TGT-3'), which is diluted in sodium saline citrate (ssc) buffer at two very different concentrations (50 pmol and 250 nmol). In the hybridization process of Bio

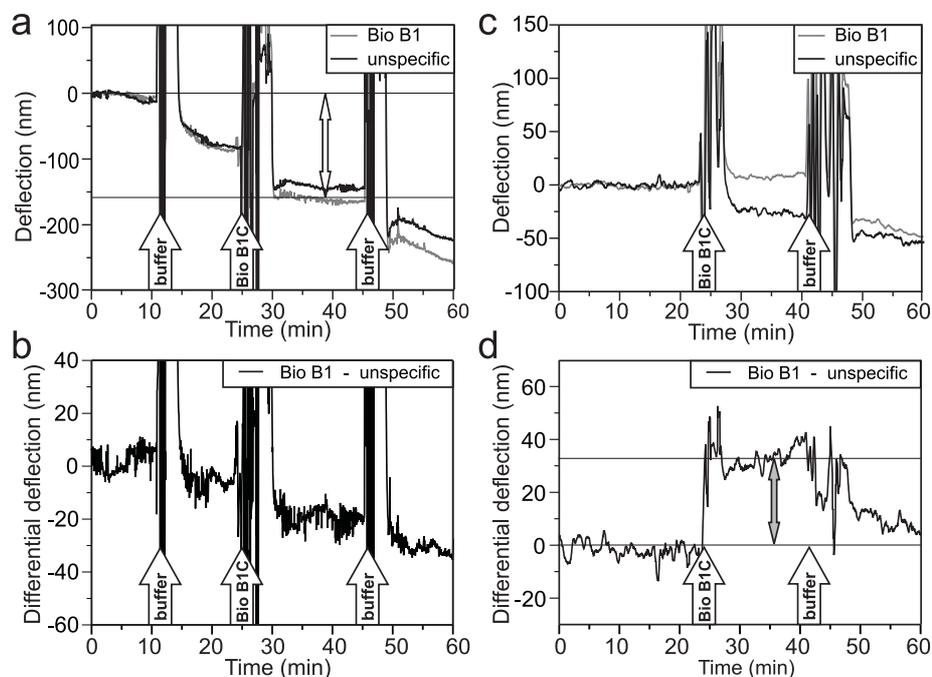


Figure 5. (a) Absolute deflection traces of a cantilever coated with Bio B1 probes and a cantilever coated with unspecific probes. The arrows label injections. The white double arrow indicates the absolute deflection change upon injection of 50 pM Bio B1C target solution in ssc buffer. (b) Differential deflection data showing the difference of the responses of the cantilever with Bio B1 probes and the cantilever with unspecific probes. Apart from thermal drift we cannot deduce a signal from that hybridization experiment, even though clear absolute deflection responses were observed. (c) Absolute deflection traces of cantilevers coated with Bio B1 probes and unspecific probes, respectively. (d) Differential deflection trace revealing a clear hybridization signal of about 35 nm (grey double arrow) upon injection of 250 nM Bio B1C target solution in ssc buffer. Please note that no fluorescent or radioactive labels were used and the hybridization was detected entirely in a nanomechanical way (data by Zhang).

B1 and Bio B1C this cantilever plays the role of the sensor cantilever. We can use any sequence as a reference cantilever that ideally shows no affinity to the target sequence Bio B1C. However, it is desirable to select a molecule for the reference cantilever that is similar to the sensor probe, for example another DNA oligonucleotide sequence of the same length, but with dissimilar bases. We selected the sequence 5'-SH-C₆-ACA CAC ACA CAC-3', which is an unspecific probe to Bio B1C for use on the reference cantilever. The experiment proceeds as follows (see figure 5(a)). First, the liquid cell containing the two cantilevers is filled with ssc buffer and we wait until a stable base line is obtained. After 11 min, ssc buffer is injected. The cantilevers deflect and we again wait until a stable baseline is reached. At 25 min the target Bio B1C is injected, which should hybridize with the Bio B1 probe, but not with the unspecific probe. Finally, we inject ssc buffer again (third injection) after 45 min. From the cantilever deflection traces shown in figure 5(a), we see that both the Bio B1 functionalized sensor cantilever (grey trace) and the unspecific reference cantilever (black trace) bend by about 80 nm, caused solely by the injection of ssc buffer. On injection of a 50 pM solution of Bio B1C in ssc buffer both cantilevers move further to an absolute deflection of 150 nm (see the white double arrow in figure 5(a)). The third injection (ssc buffer) increases the deflection of cantilevers to more than 200 nm. It appears that quite a large cantilever deflection has been measured in this experiment. But is this really a relevant signal? Several issues remain unclear in this experiment. First, why do the cantilevers move at all after injection of ssc buffer into the

liquid cell already filled with ssc buffer? Because no change of chemical environment was made, no deflection change is to be expected. Second, why do both cantilevers move on injection of Bio B1C? Only the cantilever functionalized with Bio B1 should respond to hybridization with Bio B1C by increasing the surface stress and bending. From the measured traces in figure 5(a) it is obvious that the cantilever functionalized with the unspecific probe has also responded. However, there is a difference in responses of the two cantilevers by about 20 nm. Third, why does the third injection produce further bending of both cantilevers?

Answers to these questions can be found in figure 5(b), where we plotted the numerical difference between the responses of the Bio B1 functionalized cantilever and the unspecific cantilever. The differential deflection signal is much smaller than the absolute deflection signal. This indicates that the change in absolute deflection signal is mainly caused by influences not related to the hybridization process. For example, a slightly different temperature of the injected solutions could cause thermal drifts. The difference signal is expected to show no differential deflection changes on injection of ssc buffer. During exposure to Bio B1C the probe Bio B1 hybridizes with Bio B1C, whereas the unspecific probe should not. Instead of a steplike change of differential deflection during the Bio B1C injection we observe a gradual decrease of differential deflection of about 30 nm. The most probable explanation of this finding is that thermal drift is different for each of the cantilevers, thus resulting in the gradual decrease of the differential deflection

signal. This is probably due to an inhomogeneous coating of the cantilevers. To conclude, the clear absolute deflection response observed in figure 5(a) turns out to be an artefact from a difference in temperatures of the injected solutions and the ssc buffer already present in the liquid chamber. If we had used only one cantilever in this experiment, we would have been led to the incorrect conclusion that we achieve a deflection of 150 nm from injecting a Bio B1C solution of 50 pM concentration, which would imply a major improvement in sensitivity. Actually, we can see no signal from hybridization of Bio B1 and Bio B1C in the experiment shown in figures 5(a) and (b). The set-up currently used for differential cantilever experiments is not yet sensitive enough to detect a 50 pM solution of Bio B1C target. However, if the cantilever geometry is modified (thinner cantilevers with a smaller spring constant), the sensitivity will be improved.

In a second experiment we want to investigate how another Bio B1 functionalized probe cantilever responds to injection of a 250 nM solution of Bio B1C in ssc buffer. This concentration is 5000 times higher than that used in the previous experiment. At this concentration level it should be easy to detect the hybridization response [12–14]. We decided to use another 12-mer oligonucleotide sequence from the Bio B gene, a sequence called Bio B3 (5'-SH-C₆-CCG GAA GAT TGC-3') as an unspecific probe. The liquid cell was filled with ssc buffer, and we waited until a stable baseline was reached (see figure 5(c)). After 25 min a 250 nM solution of Bio B1C in ssc buffer was injected, resulting in an absolute deflection of cantilevers in opposite directions. The absolute deflection of the Bio B1 functionalized cantilever increased by a few nanometres, whereas the absolute deflection of the unspecific cantilever decreased by about 30 nm. After re-establishing equilibrium, ssc buffer was injected at 41 min, resulting in a further decrease of the deflection to about 45 nm. Obviously, the temperature of the injected solution was not exactly the same as that of the ssc buffer initially in the liquid cell, resulting in a thermal drift superimposed on the absolute deflection signal. However, the differential deflection signal generated from the difference in responses of the Bio B1 functionalized sensor cantilever and the unspecific reference cantilever (see figure 5(d)) shows a clear steplike increase during injection of 250 nM Bio B1C solution in ssc buffer. This time the thermal drift effects superimposed on the individual absolute deflection responses of both cantilevers are cancelled out. We state that, by evaluating differential deflection data, a clear signal can be extracted from the hybridization experiment. It seems to be very important that the oligonucleotide probe layer is of a good quality and that it is homogeneous.

From the two experiments shown in figure 5, we have seen that absolute cantilever deflection data from a single cantilever is not sufficient to provide a relevant signal from a biochemical process. If one injects the target molecules to react with the corresponding probe on the cantilever, the observed bending of the cantilever might not necessarily be due to a target probe reaction. It is mandatory to use at least two cantilevers in an experiment to be able to cancel out artefacts such as thermal drifts resulting from temperature differences of the injected solutions.

The biochemical hybridization reaction discussed in this section is very specific, because it is based on a molecular

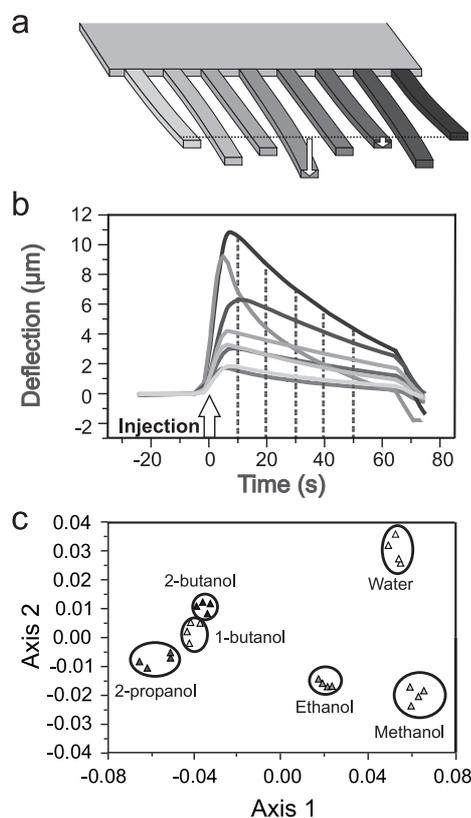


Figure 6. (a) Schematic of a cantilever sensor array used as an electronic nose. (b) Extraction of a fingerprint pattern from cantilever deflection responses. (c) Principal component analysis (PCA) plot of solvent fingerprint patterns (data by Baller).

recognition process. In the next section, we will see how the responses of an array of polymer-coated cantilevers can be used to obtain information about vapours that interact in a partially specific way with the polymer coatings.

5. Cantilever array sensors to recognize and characterize vapours

A cantilever array with eight differently coated cantilevers can be used to characterize vapours, thus working as an electronic nose. By definition an electronic nose is ‘an instrument, which comprises an array of electronic chemical sensors with partial specificity and an appropriate pattern recognition system, capable of recognizing simple or complex odours’ (Gardner and Bartlett 1992). It consists of a sensor array, a pattern classifier and a sampling system to perform measurements in a reproducible way. The sensor array is a collection of sensors exposed to the same sample and producing individual responses as well as an entire response pattern. A single chemical sensor consists of a physical transducer and a chemical interface layer or a receptor domain. The main advantages of an electronic nose are that it does not fatigue, it can be placed in hazardous environments and it is reproducible.

The sensor array presented here consists of polymer-coated cantilevers (see figure 6(a)). Various commercial polymers were dissolved in solvents (5 mg ml⁻¹). Each solution was sprayed onto one of the cantilevers of the array to obtain a chemical multisensor [5]. Detection of

vapours proceeds via diffusion of the vapour molecules into the polymer, causing the polymer to swell. The swelling in turn causes the cantilevers to bend in a way specific to the interaction between solvent vapour and polymer in terms of temporal evolution of cantilever deflections. Figure 6(b) shows the deflection traces from eight polymer-coated cantilevers upon injection of ethanol vapour during 10 s (starting at $t = 0$ s). After injection the bending signal increases, indicating that the polymer swells and bends the cantilever as the solvent molecules diffuse into the polymer layer. The pattern recognition system uses the analysis of the individual cantilever deflections at several points in time (see figure 6(b)). Signal magnitudes of all eight cantilevers at five points in time (broken lines) are extracted from the data set. These five points per cantilever sufficiently characterize the vapour desorption process during which the vapour molecules diffuse out of the polymer layer again, because the chamber is purged with dry nitrogen. Next, the five data points per cantilever are normalized to, for example, the highest magnitude of the bending response. This procedure yields a set of $8 \times 5 = 40$ normalized cantilever magnitudes, representing a so-called ‘fingerprint’ of the vapour. This constitutes the partial specificity of the chemical sensors. The data are transformed and projected using PCA algorithms. PCA extracts the most-dominant deviations in responses for various vapours. The greatest differences in signal amplitudes of the fingerprint patterns are plotted in a two-dimensional graph, whereby an individual measurement (i.e. a set of 40 normalized cantilever magnitudes) represents a single point in the PCA space. The axes refer to projections of the multi-dimensional data sets into two dimensions (principal components). This procedure is targeted at maximum distinction performance between vapours, i.e. several measurements of the same vapour should yield a cluster in principal component space, whereas measurements of differing vapours should produce well-separated clusters of measurements. Samples of various widely used polar solvents, such as water, methanol, ethanol, 2-propanol, 1- and 2-butanol, have been used to demonstrate the separation selectivity of the cantilever sensor set-up. The saturated vapour in the headspace of vials filled with 100 μl of solvent was extracted using a mass-flow controller that regulated a stream of dry nitrogen gas of 20 ml min^{-1} , which was mixed with a dry nitrogen gas stream of 20 ml min^{-1} . This mixture was then inserted into the analysis chamber [5]. The PCA evaluation of the cantilever sensor response curves is shown in figure 6(c). Clear clustering is observed for the solvents tested, demonstrating successful solvent vapour recognition and selectivity of the method. It should be noted that the ‘electronic nose’ can only recognize sample vapours that have already been measured before. It is rather a characterization tool than a chemical analysis tool. In the next section we present an application of the cantilever sensor array in medicine.

6. Medical application

The metabolic disease *diabetes mellitus* is caused by a partial or total lack of insulin. Normally, insulin promotes the entrance of glucose (sugar), the source of energy, into the cell. If insulin and hence also glucose are lacking in the cell, the body must obtain the necessary energy from fatty acids, which leads to

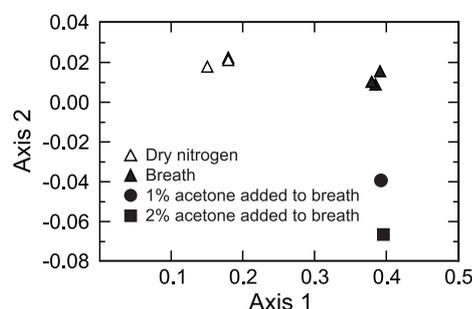


Figure 7. PCA plot of acetone detection in exhaled air (data by Schmid).

increased fat consumption. As a consequence, intermediate products, such as ketones, are produced. Acetone is one of these ketones, which can be found in exhaled air and urine. Acetone in urine can be detected using disposable test kits. The acetone in exhaled air can be detected by the physician as a putrid smell, but cannot be quantified in this way. For early diagnosis of *diabetes mellitus* it is essential to be able to detect small amounts of acetone in a patient’s breath. Here we show that the acetone content of a patient’s breath can be quantified using the cantilever array technique.

A cantilever sensor array was coated with polymers similar to the experiments discussed in the previous section. Sample bags were filled with exhaled air. 1 and 2% in volume of acetone vapour from the headspace of a closed acetone-filled vial were added to the volume of the sample bags (1000 ml), respectively. This procedure simulates a patient’s breath containing 1 or 2% acetone vapour, respectively. The experiment involved injections of 4 ml of exhaled air and vapour mixture samples with intermediate purging of the measurement chamber with dry nitrogen. The deflection pattern of the cantilevers was recorded and evaluated using PCA techniques as shown in figure 7. We observe that the dry nitrogen measurements are clearly distinguishable from the exhaled air measurements, probably because the exhaled air contains water vapour. Both the measurements with 1 and 2% in volume of acetone vapour added to exhaled air can be easily differentiated from exhaled air containing no additional acetone vapour. This study has demonstrated that cantilever array sensors are able to characterize exhaled air containing acetone vapour in a rapid and easy way.

7. Conclusions

The scanning probe methods introduced to the scientific community two decades ago have had a tremendous impact on the way surface phenomena are investigated in physics, chemistry, biology, biochemistry, engineering, technology and other disciplines. The concept of a local control interaction in combination with the technique of scanning a local probe over a surface opened up broad perspectives of new investigation techniques and applications. Using the cantilever as a platform for experiments with adsorbed molecules has advanced our understanding of processes and reactions in the nanoworld, and it has enhanced our ability to image surfaces. Cantilever sensor array techniques allow us to study physisorption and chemisorption processes, as well as to determine material-specific properties such as enthalpy

changes during phase transitions. Experiments in liquid environment have given insight into such complex biochemical reactions as hybridization of DNA or molecular recognition in antibody/antigen systems or proteomics. Detection of specific DNA oligomers against an 80-fold background of unspecific DNA has been demonstrated [14], as well as the specific detection of proteins against an excess background of unspecific proteins. This kind of cantilever based device may lead to novel concepts in nanoactuation, such as biochemically triggered valves. The goal for future developments is twofold. In terms of technological applications, new ways must be found to characterize real world samples such as clinical blood samples. The demand for medical diagnosis tools requires improvement of the sensitivity of a large number of genetic tests to be performed with single donor blood samples. There may also be completely new approaches for chemical sensors such as the use of carbon nanotubes. From a scientific point of view the challenge lies in optimizing cantilever sensors to improve their sensitivity until it reaches the ultimate limits, which may be the nanomechanical detection of individual molecules.

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