Rapid molecular detection of food- and water-borne diseases

Tiny cantilevers and lids can be used for the speedy detection of food- and water-borne pathogens. **Anja Boisen** and her colleagues believe the method will enable food and water quality control to move closer to the producer/supplier, so that any potential contamination can be discovered as fast as possible.

Micrometer-sized diving boards, also called cantilevers, can be used as highly sensitive biochemical sensors. The liquid to be analysed is passed through a pair of small cantilevers. Each cantilever has an integrated resistor with piezoresistive properties, which means that the resistance changes when the cantilever bends. Thus, by a simple electrical measurement of resistance change, the deflection of the cantilever can be determined. The measuring principle is shown schematically in Fig. 1.

One cantilever – the reference cantilever – is inert and is used to eliminate 'noise', such as temperature changes, in the system. The other cantilever is coated with a 'detector' layer that binds precisely the molecule to be detected. When the molecules land on the surface, the cantilever starts to bend due to changes in surface stress of the two faces of the cantilever. If the captured molecules, for example, tend to repel each other, the cantilever will bend downwards.

Small is good

The cantilevers can barely be seen with the naked eye. They are normally around 200 µm long, 40 µm wide and 1 µm thick, which compares with the 80 µm diameter of a human hair. The tiny size makes the cantilevers flexible and at the same time they have a high resonant frequency which makes them less sensitive to 'noise'. The cantilevers can detect deflections below 1 nm. Their small size makes a complete device with liquid handling and electronics possible in a few cm^2. Therefore, the measuring unit can be very compact and portable and suitable for 'point-of-use' analysis.

It is impossible to make advanced mechanical structures with micrometer dimensions and integrated electronics using fine mechanics. Instead, we use so-called photolithographic processes where the cantilevers are fabricated by etching a thin silicon wafer three-dimensionally. The procedure is relatively straightforward and is highly suitable for mass production. Therefore, it might be possible to make sensors so cheaply that they can be disposable. Fig. 2 shows an image of two silicon cantilevers.

Plastic is the future

The cantilevers' sensitivity relies on their flexibility. Therefore, we have started to use polymers which are 40 times softer than silicon. In this way the sensitivity is immediately improved and the manufacturing cost is at the same time significantly reduced. The cantilevers are made by spin-coating the polymer onto a silicon carrier wafer. The polymer is structured by UV-lithography and by successive coating and structuring steps, cantilevers placed in micrometer-sized channels can be produced. An example of a polymer cantilever with an integrated gold resistor is shown in Fig. 3.

Catching bacteria

Whole bacteria or parts of bacteria can be caught directly on the cantilever. For example, if the cantilever is coated with antibodies against *Escherichia coli* it will specifically bind that organism and the cantilever will only bend if *E. coli* is present in the sample. The sensor can easily be expanded to contain several cantilevers, each coated with a specific 'detector' molecule. In this way it is possible to detect multiple bacteria simultaneously.

The cantilevers can also be used to recognize DNA. A single stand of DNA from *E. coli* is placed on a cantilever. A water or food sample can be treated relatively quickly so that the DNA from the bacteria is released and split into single strands. Next, the pretreated sample is sent to the cantilevers which will only react if the single-stranded DNA on the cantilever matches the

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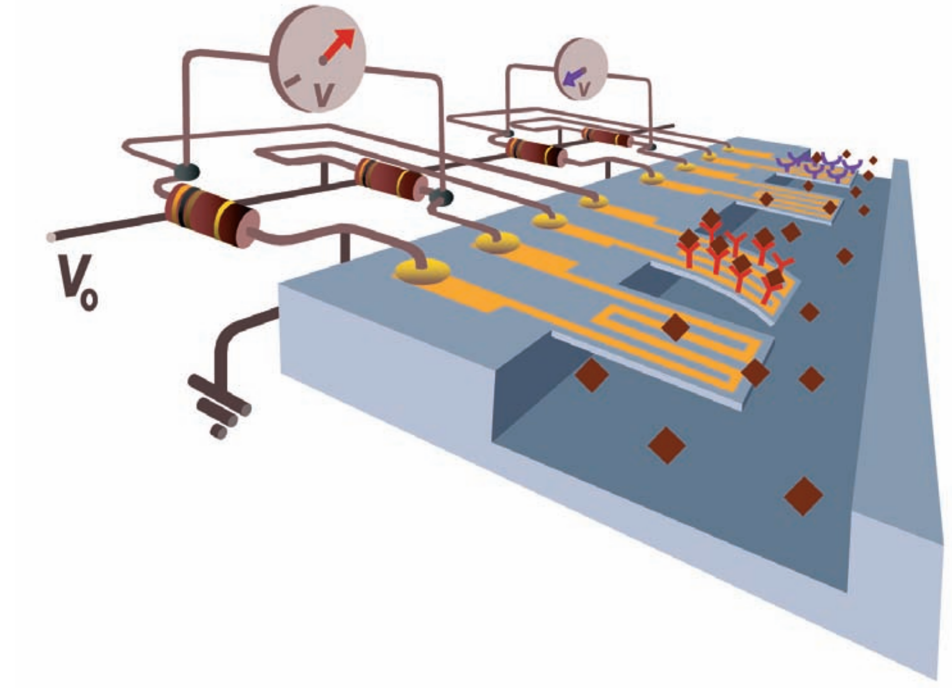

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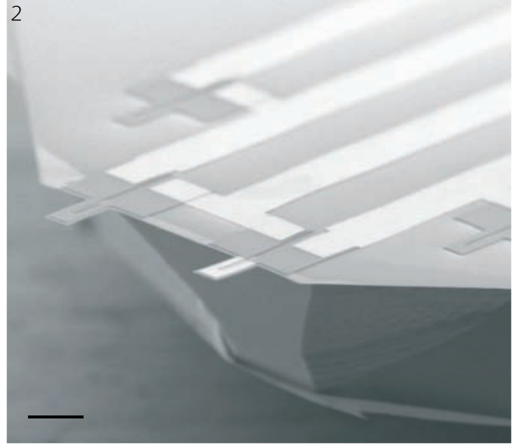

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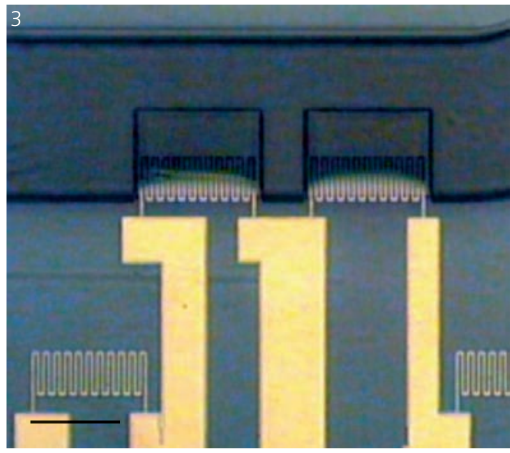

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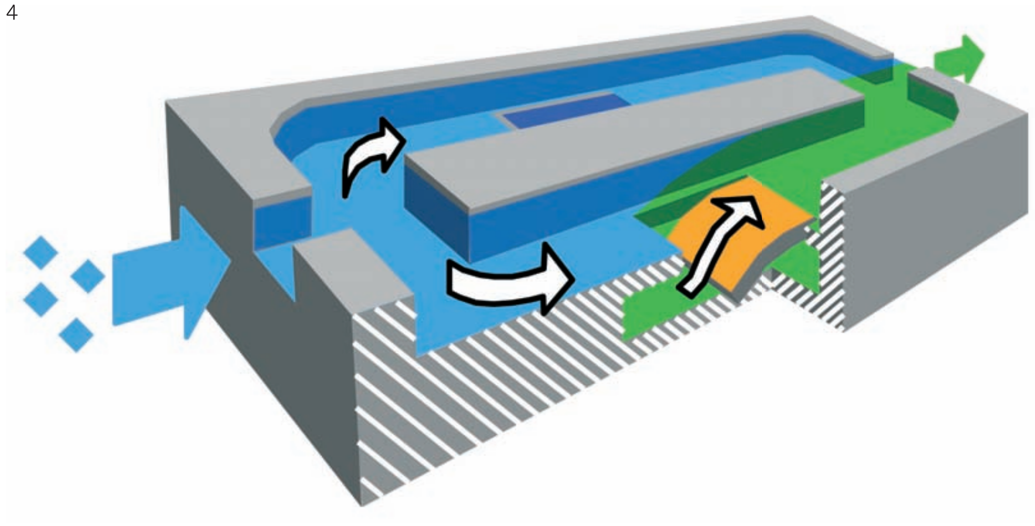
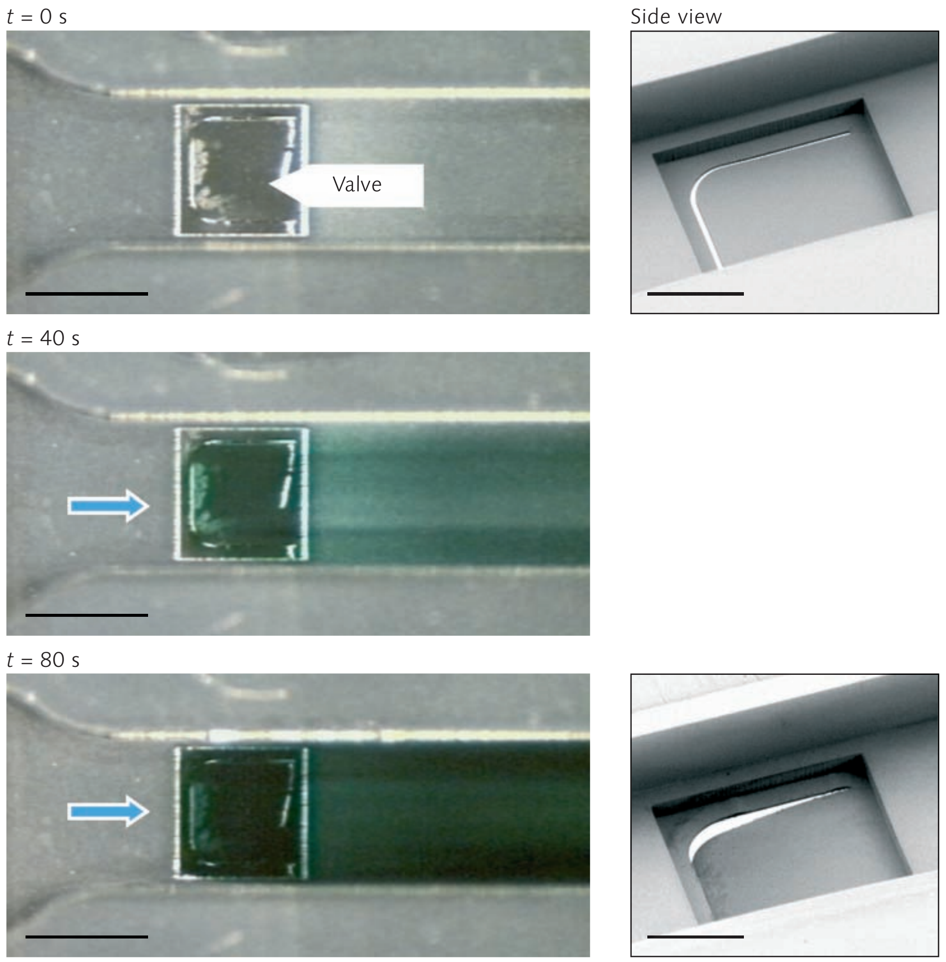


▲ Fig. 1. Schematic drawing of the cantilever measuring principle. When molecules attach to the cantilever, the cantilever bends and the bending is detected as a change in the resistance of the resistor placed inside the cantilever.

▲ Fig. 2. Image of two silicon cantilevers. One cantilever is coated with a thin layer of gold for the specific binding of molecules with a sulfur group at the end. Sulfur binds almost covalently to gold. Bar, 100 µm.

▲ Fig. 3. Image of a polymer cantilever with integrated gold resistors. Bar, 200 µm.

▲ Fig. 4. Schematic drawing of the operating principle of the 'lid' sensor. As molecules bind to the orange lid, the lid bends and releases the green colour.



◀ Fig. 5. Proof of principle of the 'lid' device. As a thin layer of aluminium is etched away from the lid's top surface, the lid starts to deflect and a green marker solution is released from the reservoir below the lid. Bars, 0.5 mm (main views), 200 μm (side views).

single-stranded DNA in the solution. If the cantilever bends, then *E. coli* is present in the sample.

The lid device

A new sensor principle, which we call the lid device, is illustrated in Fig. 4. Coloured marker molecules are loaded in a small container closed by a flexible lid. The lid is coated with specific detector molecules which bind the molecules under investigation. This binding causes the lid (just like a cantilever) to deflect, the marker molecules are released and they can be detected by the naked eye. The complete sensor is approximately 1×1 cm in size and is made of plastic. The deflection of the lid can also be caused by removal of a material on the upper surface of the lid. An example of the removal of a thin metal layer on the top of the lid and the subsequent colour change is shown in Fig. 5. The removal of material can be caused by bacterial activity (the bacteria 'eat' food on the lid) and the sensor can thus be used to monitor the presence/activity of bacteria.

The lid device is based on the release of a colour which can easily be detected by the eye following a specific reaction. This principle could be used in food diagnostics where there is a great need for cheap, disposable sensors. The sensor could be included in food packaging since it requires no external energy and is cheap to make. When a food is infected, the control unit in the plastic wrapping becomes coloured. Thus a simple colour indicator can show the quality of the food.

Only our imagination limits the future

There are many other possible applications for the technology. The detection of DNA can also be used to look for human gene-related diseases. For example, specific DNA sequences are known to be related to a larger risk of developing breast cancer. One can also imagine using the sensor in environmental control for monitoring the quality of drinking water. The cantilevers allow us to monitor the binding of molecules 'on-line' and it is therefore possible to follow bacteria-, enzyme-, DNA- and virus- binding processes in real time. Moreover, the lid device opens up the production of autonomous, disposable systems which enable the end-user to control water as well as food quality.

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