

Design & fabrication of cantilever array biosensors

Surface immobilization of functional receptors on microfabricated cantilever arrays offers a new paradigm for the development of biosensors based on nanomechanics. Microcantilever-based systems are capable of real-time, multiplexed detection of unlabeled disease markers in extremely small volumes of samples. Currently available fabrication technology will allow the integration of electronic readout and sample introduction into a single unit, decreasing the device size, detection time, and cost. Biosensing technologies based on microfabricated cantilever arrays involving multiple cantilevers, electronic processing, and even local telemetry on a single chip have the potential of satisfying the need for highly sensitive and selective multiple-target detection in very small samples. Here we will review the design and fabrication process of cantilever-based biosensors.

Anja Boisen¹ and Thomas Thundat²

¹Technical University of Denmark, Anker Engelds Vej 1, Building 101A, 2800 Kgs, Lyngby, Denmark

²Oak Ridge National Laboratory, 1 Bethel Valley Road, Oak Ridge, TN 37831-6123, USA

Here we discuss the design and fabrication of microfabricated cantilever arrays as a potential platform for multiplexed detection of biomarkers in real time with extremely high sensitivity and selectivity. Because of their versatility, cantilever array sensors can be used for receptor-based and label-free detection of biomolecules and integrate well into a multiplexed sensor system.

The need for miniature biosensors for multiple-analyte detection

Since most disease biomarkers are not sufficiently selective, detection of a single biomarker provides only limited specificity and cannot

be used for positive identification of a disease. To overcome this challenge, multiple tests extended over time are often carried out for positive disease identification. Health professionals often rely on multiple, discrete tests carried out with different and spatially distributed equipment for analysis and identification. This process leads to unacceptable time delays, costs, and inconvenience. This is especially true for complex diseases such as cancer. Highly reliable disease markers are often absent in complex diseases, requiring the health professional to depend on detection of multiple markers for decreasing false positives. Since early detection is key for successful treatment and for decreasing the mortality rate, development of a

single device capable of detecting multiple biomarkers in very small concentrations in the presence of a very high background of similar molecules is essential. Unfortunately, currently available sensor platforms fail to satisfy the conditions of sensitivity, selectivity, simultaneous detection of multiple targets, cost, and the ability to use small-volume samples. However, biosensing technologies based on microfabricated cantilever arrays have the potential to satisfy all the stringent conditions for highly sensitive and selective multitarget detection in very small sample volumes.

Microfabricated cantilever array sensors have attracted considerable attention over the last decade because of their potential as a highly sensitive platform for high-throughput and multiplexed detection of chemicals and biomolecules¹⁻¹². Like miniature diving boards, microcantilevers are mechanical beams that are anchored at one end. They can be readily fabricated from silicon wafers and other materials. Their typical dimensions are approximately 100 μm long, 20 μm wide, and 1 μm thick. (In comparison, the diameter of a human hair is approximately 80 μm .) Microcantilevers are extremely sensitive displacement sensors; they can even detect forces involved in molecular adsorption. As we shall see later, this nanoscale deflection is caused by a variation in the cantilever surface stress due to changes in the adsorption-induced free energy. The bending of the cantilever beam can be measured electrically by a piezoresistive readout technique that allows integration into a miniature device. Biological specificity in detection is typically achieved by immobilizing selective receptors or probe molecules on one side of the cantilever surface. A cantilever beam with immobilized receptors undergoes bending when it is bathed in fluid and target molecules are introduced into the fluid. The extent of the cantilever bending is a function of the number of target molecules binding to the immobilized receptors on the cantilever surface.

Cantilever sensors detect the presence of unlabeled biomarkers in fewer processing steps than is required by currently available diagnostic platforms, such as enzyme-linked immunosorbent assays for proteins or microarrays for nucleic acids. Since cantilevers can be fabricated into arrays for simultaneous detection of multiple analytes, the detection time can be significantly shortened, resulting in substantial savings in cost and time. High sensitivity in detection will eventually lead to early detection of many complex diseases, decreasing severity, complications, and mortality rates. Ultimately, all of these advantages will reduce assay costs and hence costs to the patient and healthcare providers. Since microfabrication techniques offer mass production of miniature sensors, microcantilever arrays offer a clear path to the development of cost-effective sensors with unprecedented sensitivity for biosensing applications.

Microcantilever sensors can also be operated in resonance frequency mode where mass loading due to molecular adsorption is detected as changes in resonance frequency. Many bioanalytes have been successfully detected using the resonance frequency method¹³⁻¹⁶.

However, resonance frequency damping in liquids makes the resolution of frequency measurements poor due to low quality factor¹⁷⁻¹⁹.

Mechanics of cantilever deflection

Like liquids, solids have surface tension, often called "surface free energy." Unlike the molecules in a liquid surface, which are free to move, the atoms on a solid surface are fixed in a lattice structure. Adsorption of molecules on a solid surface is primarily driven by the tendency toward minimization of energy (molecular adsorption decreases the surface free energy of the solid surface). If the adsorption of molecules on the surface of a thin material is restricted mostly to one side, for example, by making the opposite surface inert, a differential surface stress is generated between the two surfaces. This differential surface stress causes the material to deform. This effect can be easily observed with a microcantilever beam. Surface stress, σ , and surface free energy, γ , can be related using the Shuttleworth equation²⁰:

$$\sigma = \gamma + \frac{d\gamma}{d\epsilon} \quad (1)$$

where, the surface strain $d\epsilon$ is defined as the ratio of the change in surface area to the total area, $d\epsilon = \frac{dA}{A}$. For liquids, the second term is zero.

Since surface stress and surface free energy are related, a change in surface stress follows molecular adsorption. The differential surface stress created by molecular adsorption results in cantilever bending. Stoney's equation²¹ relates the difference in surface stress, $\Delta\sigma$, between the chemically modified surface and the untreated surface with the cantilever deflection, Δh :

$$\Delta h = \frac{4(1-\nu)L^2}{Et^2} (\Delta\sigma_1 - \Delta\sigma_2), \quad (2)$$

where ν is the Poisson's ratio of the material, E is Young's (elastic) modulus of the cantilever material, and t and L are the thickness and the length of the cantilever, respectively. By optimizing the length and thickness, it is possible to make a cantilever very sensitive to small changes in surface free energy. Unlike a clean surface in a vacuum, a surface exposed to ambient air has adsorbed molecules occupying adsorption sites. When exposed to target molecules, the target molecules either adsorb onto the molecules that are already adsorbed on the surface or replace them²²⁻²⁵. Therefore, the surface energy change determined from experiment may not readily match the theoretical values.

Origins of parasitic deflections

Secondary effects, such as adsorption-induced changes in surface charge density, creation of ionized species resulting from receptor analyte interaction, or swelling of the immobilized receptor layers,

can also cause a cantilever to deflect. As long as there is a differential response from opposite sides of the cantilever, the cantilever deflects. Often, the resultant deflection is due to a combination of many effects that may be taking place on the cantilever surfaces. Physical variables such as changes in temperature, flow rate, pH, and ionic concentration may result in cantilever deflection²⁶.

Since cantilevers may be bimaterial in construction, changes in the coefficients of thermal expansions may cause bending with changes in temperature. A cantilever also undergoes bending due to flow-induced venturi forces. Materials such as silicon oxide and silicon nitride have surface species that ionize depending on the pH of the solution and can also cause bending. To eliminate the effects introduced by these artifacts, reference cantilevers are often fabricated into the arrays (paired with the sensor cantilevers), and deflection is measured in a differential mode with respect to the reference cantilever.

Electronic monitoring of cantilever deflection

The motion of a cantilever beam can be sensitively monitored by means of a variety of techniques, such as variations in optical beam deflection²⁷, embedded transistor semiconductivity²⁸, piezoelectricity²⁹⁻³⁰, and piezoresistivity³¹⁻³². All the various signal transduction methods have advantages and disadvantages based on the application. In optical beam deflection, the cantilever motion is detected by reflecting a focused beam of light from the tip of a cantilever into a position-sensitive detector. Although the method based on optical beam deflection has been widely used in designing sensors, the sensors are neither compact nor cost-effective. In the embedded transistor technique, a metal oxide semiconductor field-effect transistor (MOSFET) is embedded at the base of the cantilever. The stress from the bending of the cantilever changes the carrier mobility and drain current of the MOSFET. The embedded transistor technique has not been studied extensively. In the piezoresistive technique, the resistance of an asymmetrically doped cantilever varies as a function of bending. The piezoresistive approach has been widely studied as a signal transduction method for biosensor applications. The piezoresistive method is compatible with microfabrication and miniaturization³³. In the next section we will discuss the details of piezoresistive cantilever fabrication.

Design and fabrication of piezoresistive cantilever arrays

Piezoresistivity is a property of certain conductive materials, such as doped silicon, whose electrical resistance varies as a function of applied strain. When the piezoresistive technique is applied to monitor cantilever deflection, the cantilever is fabricated with an integrated resistor having piezoresistive properties, and its resistance changes as a function of cantilever bending. The extent of cantilever bending can be sensitively measured by a simple electrical measurement³⁴⁻³⁶.

The commonly fabricated piezoresistors are based on boron-doped silicon layers. The doped silicon layers are then capped with insulating materials. To enhance sensitivity, the piezoresistive layer of the cantilever should be placed in the region of maximum stress, preferably on the surface. For a first approximation, the resulting relative change in resistance is directly proportional to the stress, and therefore, to the deflection.

In general, variations in cantilever resistance are measured with respect to a reference cantilever fabricated adjacent to the sensor cantilever. The measuring principle is shown schematically in Fig. 1. The reference cantilever is inert and is used to eliminate noise in the system, such as temperature changes. Unlike the reference cantilever, the sensor cantilever is coated on one side with a "detector" layer that specifically binds with the molecule of interest. Generally, the side opposite to the functionalized layer is made biologically inert using polyethylene glycol to avoid any unspecific adsorption. When the molecules bind onto the coated surface, the cantilever starts to bend due to the differences in surface stress between the two faces of the cantilever. Typically, the sensor cantilever is connected to the reference cantilever by two external resistors to form a Wheatstone bridge configuration. In that way, an output signal is only recorded when there is a difference in the deflection of the two cantilevers, and thus noise is reduced significantly. Fig. 2 shows a chip with two microfabricated cantilevers for differential measurements.

Their tiny size makes the cantilevers flexible; at the same time, they have a high resonant frequency, which makes them less sensitive to external vibration. The silicon resistor is defined in microcrystalline or single crystal silicon and encapsulated in silicon nitride. The thickness of the deposited silicon nitride on either side of the cantilever is adjusted so that the neutral axis of the cantilever lies inside the silicon

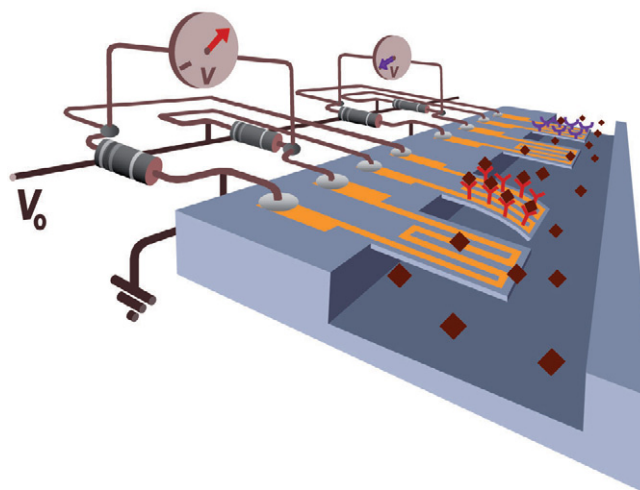


Fig. 1 Schematic drawing of the cantilever measuring principle. When molecules attach to the cantilever, the cantilever bends and the bending is detected as a change in the resistance of the resistor placed inside the cantilever. A measurement is always performed on two cantilevers simultaneously. One cantilever serves as reference and only the differential signal is recorded. (Courtesy of Daniel Häfliger).

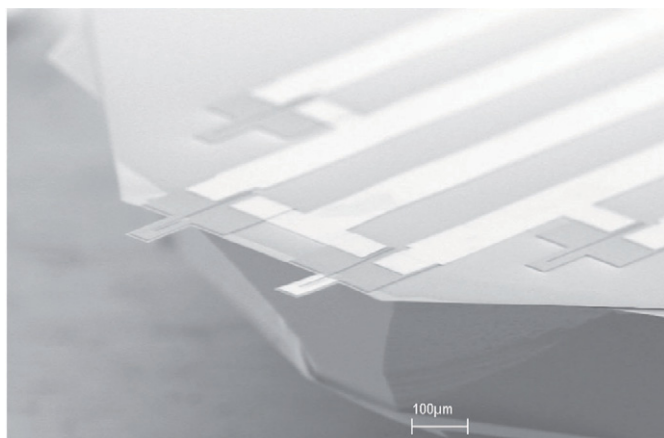


Fig. 2 Image of two silicon cantilevers. One cantilever is coated with a thin layer of gold for the specific binding of molecules with a sulfur group at the end. Sulfur binds strongly to gold. (Courtesy of Jacob Thaysen)

nitride layer rather than in the silicon layer. This asymmetry in material composition ensures that the resistor is placed close to one of the cantilever surfaces for optimal sensitivity. In addition, the silicon nitride serves as efficient electrical insulation for the resistor and ensures that the device can be operated in liquids. The signal-to-noise ratio of the piezoresistive cantilever depends highly on the doping and the crystallinity of the silicon layer. The best performance is clearly found for single crystalline resistors, which can detect deflections below 1 nm resolution³⁷.

It is impossible to fabricate advanced mechanical structures with micrometer dimensions and with integrated electronics using fine mechanics. Instead, cleanroom-based processes are used, in which the cantilevers are fabricated by three-dimensional etching of a thin silicon wafer. Low-pressure chemical vapor deposition is used to deposit the silicon nitride layers. It is easy to produce different geometries and different layouts of the cantilevers, and they can be integrated with channels for liquid handling. In Fig. 3, an image of 32 cantilevers placed in a channel is shown. The channel is used to guide the liquids under investigation to the cantilevers. The small size of the cantilevers makes it possible to realize an integrated device with liquid-handling capability and readout electronics on a few square centimeters.

The flexibility of a cantilever determines its sensitivity to surface stress. Thus cantilevers fabricated from polymers, being more flexible, offer better deflection sensitivity. For example, it is possible to fabricate a cantilever from the epoxy-based polymer SU-8, which is 40 times softer than silicon³⁸⁻³⁹. In this way, sensitivity is immediately improved and the fabrication cost is at the same time significantly reduced. The polymer cantilevers are fabricated by spin-coating the polymer onto a silicon carrier wafer that has been treated with an anti-stiction coating. The polymer is structured by ultraviolet lithography and by successive coating and structuring steps. Cantilevers can be placed inside micrometer-sized channels. At the end of the processing, the cantilevers are simply removed from the substrate with tweezers.

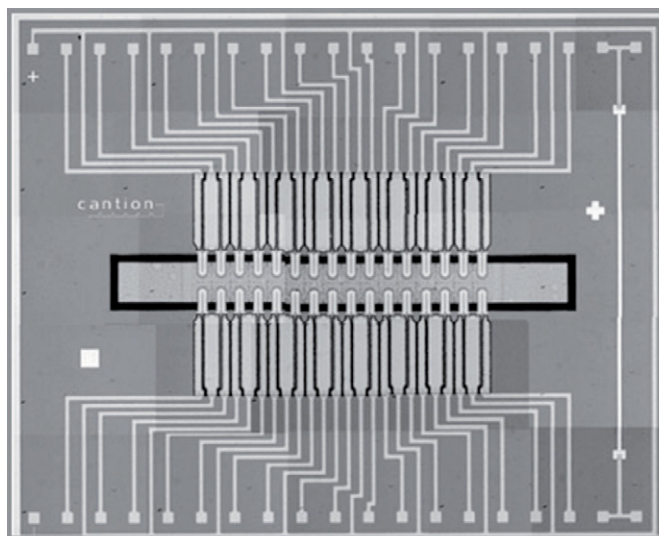


Fig. 3 An array of 32 piezoresistive cantilevers arranged in a microfluidic channel (Courtesy of Jacob Thaysen).

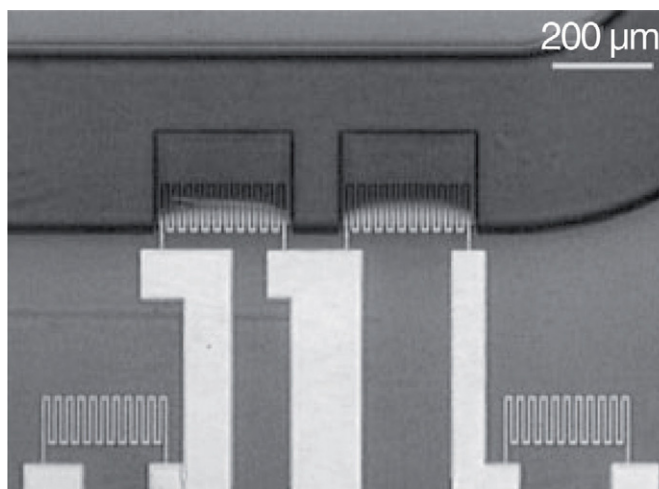


Fig. 4 Image of polymer (SU-8) cantilevers with integrated gold resistors. These devices are softer than similar silicon devices and are much faster to fabricate. However, polymers with the same stability as silicon still need to be identified. (Courtesy of Alicia Johansson).

An example of a polymer cantilever with integrated gold resistor is shown in Fig. 4. The gold resistors serve as low-noise piezoresistors; the signal-to-noise ratio is comparable to the value for silicon nitride cantilevers with single crystal resistors⁴⁰. The relative resistance change of gold is approximately 40 times smaller than for single crystal silicon, and new materials are therefore being investigated as possible candidates for even better piezoresistors. The challenge is to find a material that is soft while having a large change in resistance upon deflection. Moreover, the electrical noise in the system should be low. Materials such as polyaniline, Poly(3,4-ethylenedioxythiophene) (PEDT), and SU-8 doped with 20 nm carbon particles have been investigated.

Microcantilever biosensors

Microcantilever beams are very sensitive displacement sensors. Since they do not have any intrinsic chemical or biological selectivity, highly selective receptors are often immobilized on the cantilever surface for achieving selectivity in detection. Therefore, the selectivity of detection depends on the selectivity of the receptor-analyte interaction. Many approaches have been used to immobilize the molecular recognition agents to a microcantilever sensor. A commonly used method involves using thiol chemistry to attach receptors. Cantilevers are first coated, using an e-beam evaporator, on one side with 2 to 3 nm of chromium followed by 25 to 30 nm of gold. The chromium acts as an adhesion layer for the gold. Silane chemistry can be used for immobilizing receptors on silicon oxide cantilevers. For thiol self-assembled monolayers (SAMs) and organosilane modification, dip-coating is the preferred method for immobilization of receptors to achieve high grafting density. The SAM is self-limited to coverage of a monolayer on a gold film. The organosilane coatings also are of the order of a monolayer but can become multilayered upon extended exposure to the solution. The functionalized surfaces are freshly prepared for each experiment. The reliability of microcantilever-array-based detection is increased by having several sensors operating in parallel. Such increased reliability is especially important for biomedical applications. Microcantilever arrays fabricated in groups can also be used for simultaneous detection of multiple biomarkers.

The nanomechanical detection of biomolecules using cantilever arrays offers enormous possibilities for label-free biosensing. Various biomolecules have been detected using cantilever platform⁴³⁻⁶¹. The simplest biosensor demonstration would be detection of DNA strands. Thiol chemistry is used to immobilize single-stranded DNA molecules (DNA probes) on the gold-coated side of the

cantilever. The single-stranded DNA immobilization process itself results in cantilever bending. Exposing the cantilever to a stream of complementary single-stranded DNA (target DNA) results in hybridization and further bending. Fig. 5 shows cantilever bending due to DNA hybridization. The response of the cantilever due to hybridization reaches a steady state when all the DNA probes are hybridized. The same concept can also be used for detecting proteins. Fig. 6 shows cantilever bending as a function of time due to specific adsorption of c-reactive proteins (CRP) on a cantilever with immobilized antibodies. In this case the cantilever is first functionalized with CRP antibodies. The antibody-antigen interaction causes the cantilever to bend. It should be noted that most biomolecular binding are irreversible at room temperature.

Recently implantable biosensors have been fabricated using cantilever platform⁶²⁻⁶³. Since regeneration of a biosensor is very challenging, the implanted sensors were used for detection of blood gases, for example blood alcohol. In this case the cantilevers were coated with hydrophobic coating on the backside and a polymer thiolated siloxane/fluoro alcohol (TSXFA) on the gold side of the cantilevers. The TSXFA coating absorbs alcohol differentially creating a cantilever bending. Other polymers with higher partition function for alcohol such as methyl phenyl mercapto propyl silicone (OV17 MCP20) were also used as selective layer. An implantable sensor for short-term *in vivo* detection of blood alcohol level using cantilevers with integrated readout and telemetry developed by Ferrell *et. al.* is shown in Fig. 7. The *in vivo* detection using implantable sensors is a formidable challenge due to many current technological limitations. For example, at present there are no highly selective receptors that can work reliably and reversibly *in vivo*. Therefore, regenerating the sensor device after detection, and keeping the efficacy of the receptors

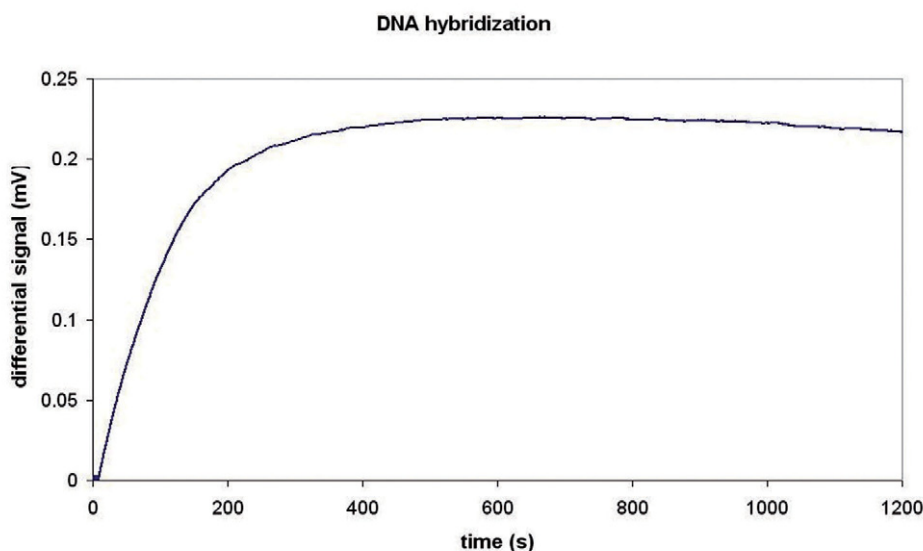


Fig. 5: Detection of DNA hybridization using a cantilever immobilized with single-stranded DNA. The figure shows cantilever bending when complementary single-stranded DNA solution was introduced into the flow.

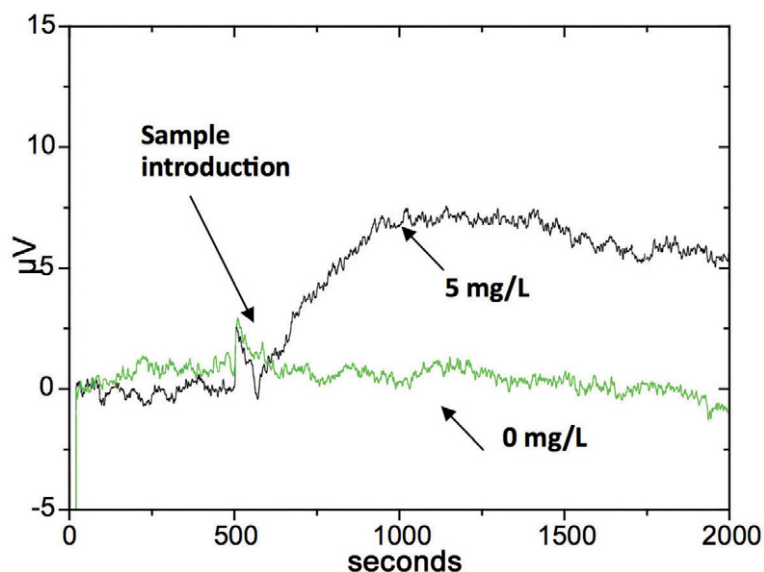


Fig. 6: Detection of C-reactive protein using a piezoresistive cantilever immobilized with CRP-antibody.

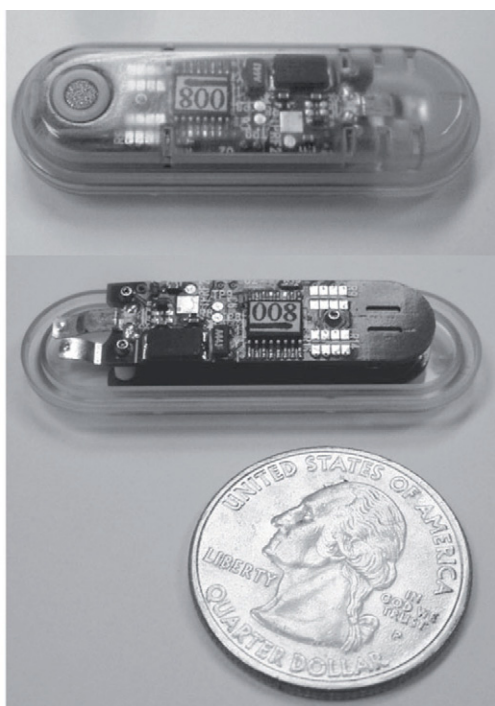


Fig. 7: An integrated implantable sensor with wireless telemetry for detecting blood gases. The figure shows closed and open implantable telesensor capsules alongside a U.S. quarter coin. A titanium frit is seen in the upper portion as the circular membrane on the left side of the closed capsule. Under the frit is a membrane filter for partial separation of the analyte from the interstitial fluid. This covers two arrays of four piezoresistive microcantilever sensors that are connected in a set of Wheatstone bridges. The signals are delivered to a sigma-delta ADC and thence to a very low-power microprocessor. This is programmed to allow modulation of the transmissions to a nearby receiver/computer combination where the data are displayed graphically. In the present embodiment, the telesensor capsule can transmit both chemical data and body temperature every five minutes for six weeks (Courtesy of T.L. Ferrell)

for extended periods of time *in vivo* are also major issues that need to be addressed. Also, potential biofouling of the sensor surface and the biocompatibility of the chemical interface for target-analyte adsorption pose challenges.

Challenges

The technology for designing and fabricating cantilever arrays with electronic readouts is well advanced. However, despite the many advantages of microfabricated cantilever-based sensor arrays, a number of challenges remain to be overcome before they can find a widespread market. Just as similar challenges have been met by the semiconductor electronics industry, we believe these challenges will be overcome. Integration of cantilever arrays and microfluidic channel networks is still under development. Receptor immobilization on individual cantilevers in an array in a reproducible fashion from chip to chip is still remains as a challenge. Novel robust receptor immobilization techniques that can work under solution in a reproducible manner as well as ways by which receptors are applied on the cantilever need to be optimized⁶⁴⁻⁶⁶. Currently, the performance characteristics variation from array to array due to variation in surface density of immobilized receptors is not at an acceptable level for marketing. The shelf life of the immobilized receptors and effect of the environment on their performance need to be investigated. Techniques for sample collection and injection into the sensor arrays are also needed to facilitate applications. The role of various cantilever parameters, such as crystalline nature of the coating materials, need to be investigated^{67,68}. Also needed is a better understanding of the biomolecular adsorption process on the cantilever⁶⁹.

Experimental results from our laboratories as well as from laboratories around the world show that multiple-analyte detection


from a raw sample may not be achieved with present technologies. Therefore, integration of some rudimentary on-chip sample processing is required prior to detection by cantilever arrays. Such on-chip processing can be incorporated without increasing the chip size or power requirements using presently available techniques.

Concluding remarks

While unprecedented sensitivity and receptor-based selectivity have been demonstrated for microcantilever biological sensors, the performance reliability is not consistent due to problems associated with reliable and reproducible immobilization of receptors. The deposition and immobilization of the biologically and chemically selective coatings appears to be a critical issue affecting consistency

and reproducibility. Once these challenges are overcome, there exist a number of consumer applications ranging from the health care industry to food safety to environmental management. This will be especially true where multiple analytes need to be detected.

Acknowledgments

We would like to thank our colleagues and collaborators cited in this review for their contributions. We are indebted to Jacob Thaysen, Sangmin Jeon, Seonghwan Kim, Larry Senesac, and T.L. Ferrell for help with figures. T. Thundat would like to thank the DOE Office of Science, Biological and Environmental Research, for its support. Oak Ridge National Laboratory is managed by UT-Battelle under contract No. DE-AC05-00OR22725. 

REFERENCES

- Gimzewski, J. K., et al., *Chemical Physics Letters* (1994) **217**, 589.
- Thundat, T., et al., *Appl. Phys. Lett.* (1994) **64**, 2894.
- Chen, G. Y., et al., *J. Appl. Phys.* (1995) **77**, 3618.
- Butt, H. J., *J. Colloid Interface Sci.* (1996) **179**, 251.
- Thundat, T., et al., *Microscale Thermophys. Eng.* (1997) **1**, 185.
- Berger, R., et al., *Science* (1997) **276**, 2021.
- Sepaniak, M. J., et al., *Analytical Chemistry* (2002) **74**, 568A.
- Ziegler, C., *Analytical & Bioanalytical Chemistry* (2004) **379**, 946.
- Lavrik, N. V., et al., *Rev. Sci. Instrum.* (2004) **75**, 2229.
- Fritz, J., *The Analyst* (2008) **133**, 855.
- Datar, R., et al., *MRS Bulletin* (2009) **34**, 449.
- Khaled, A. R. A., et al., *Sensors and Actuators B* (2003) **94**, 103.
- Ilic, B., et al., *Appl. Phys. Lett.* (2004) **85**, 2604.
- Gupta, A., et al., *Journal of Vacuum Science & Technology B* (2004) **22**, 2785.
- Gupta, A., et al., *Applied Physics Letters* (2004) **84**, 1976.
- Su, M., et al., *Applied Physics Letters* (2003) **20**, 562.
- Oden, P. I., et al., *Appl. Phys. Lett.* (1996) **68**, 3814.
- Sader, J. E., *J. Appl. Phys.* (1998) **84**, 64.
- Chon, J. W. M., *J. Appl. Phys.* (2000) **87**, 3978.
- Shuttleworth, R., *Proc Phys Soc (London)* (1950) **A63**, 444.
- Stoney, G. G., *Proc. R. Soc. London, Ser* (1909) **A82**, 172.
- Raiteri, R., and Butt, H. -J., *J. Phys. Chem.* (1995) **99**, 15728.
- Raiteri, R., et al., *Electrochim. Acta.* (2000) **46**, 157.
- Raiteri, R., et al., *Sens. Actuators B, Chem. (Switzerland)* (2001) **B79** 115.
- W. Haiss, *Rep. Prog. Phys.* 64, (2001) p. 591.
- Ji, H. F., et al., *Sens. Actuators B, Chem. (Switzerland)* (2001) **B72**, 233.
- Meyer, G., and Amer, N. M., *Appl. Phys. Lett.* (1988) **53**, 1045.
- Shekhawat, G., et al., *Science* (2006) **311**, 1592.
- Lee, S. S., and White, R. M., *Sens. Actuators A* (1998) **52**, 41.
- Lee, J. H., et al., *Integr. Ferroelectr.* (2002) **50**, 43.
- Tortonesi, M., et al., *Solid-State Sensors and Actuators (Transducers '91)* (1991) 448.
- Boisen, A., et al., *Ultramicroscopy* (2000) **82**, 11.
- Madou, M., *Fundamentals of Microfabrication* CRC Press (1997).
- Thaysen, J., et al., "Cantilever-Based Bio-Chemical Sensor Integrated in a Microliquid Handling System" Technical Digest. MEMS: 14th IEEE International Conference on Micro Electro Mechanical Systems (2001) **40**.
- Rasmussen, P. A., et al., *Appl. Phys. Lett.* (2005) **20**, 203502.
- Choudhury, A., et al., *Journal of Micromechanics and Microengineering* (2007) **17**, 2065.
- Please see www.cantion.com.
- Johansson, A., et al., *Sensors and Actuators A* (2005) **123**, 111.
- Johansson, A., et al., *Appl. Phys. Lett.* (2007) **17**, 173505.
- Johansson, A., *SU-8 Cantilever sensor with integrated read out*, Ph.D Thesis, D.T.U. (2006).
- Mateiu, M. et al., *Microelectron Eng.* (2007) **84**, 1270.
- Lillemoose, M., et al., *Composite Science and Technology* (2008) **68**, 1831.
- Fritz, J., et al., *Science* (2000) **288**, 316.
- Hansen, K. M., et al., *Anal. Chem.* (2001) **73**, 1567.
- Mukhopadhyay R., et al., *Langmuir.* (2005) **21** (18), 8400.
- Álvarez M., et al., *Langmuir* (2004) **20** (22), 9663.
- Savran C. A., et al., *Analytical Chemistry* (2004) **76** (11), 3194.
- Wu, G., et al., *Nature Biotechnol* (2001) **19**, 856.
- Wu, G., et al., *Proc. Natl. Acad. Sci. U.S.A.* (2001) **98**, 1560.
- McKendry, R., et al., *Proc. Natl. Acad. Sci. U.S.A.* (2002) **99**, 9783.
- Arntz, Y., *Nanotechnology* (2003) **14**, 86.
- Zhang, J., et al., *Nature Nanotechnol.* (2006) **1**, 214.
- Yue, M., et al., *Nano. Lett.* (2008) **8**, 520.
- Zhang, J., et al., *Nature Nanotechnology* (2006) **1** (3) , 214.
- Dhaval, B., et al., *Journal of the American Chemical Society* (2006) **128** (11), 3716.
- Mertens, J., et al., *Nature Nanotechnol.* (2008) **3**, 301.
- Rasmussen, P. A., et al., *Ultramicroscopy* (2003) **97**, 371.
- Arntz, Y., et al., *Nanotechnology* (2003) **14**, 86.
- Dutta, P., et al., *Analytical Chemistry* (2003) **75**, 2342.
- Mukhopadhyay, R., et al., *Nano Letters* (2005) **5**, 2385.
- Weeks, B.L., et al., *Scanning* (2003) **25**, 297.
- Cheney, C. P., et al., *Sensors and Actuators B* (2009) **138**, 264.
- Cheney, C. P., et al., *Appl. Phys. Lett.* (2007) **90**, 013901.
- Kohale, S., et al., *Langmuir* (2007) **23**, 1258.
- Pinnaduwa, L. A., et al., *Langmuir* (2003) **19**, 7841.
- Bietsch, A., et al., *Nanotechnology* 2004 **15** (8), 873.
- Godin, M., et al., *Langmuir* (2004) **20**, 7090.
- Tabard-Cossa, V., et al., *Anal. Chem.* (2007) **79**, 8136.
- Hagan, M. F., et al., *Journal of Physical Chemistry B.* (2002) **106**, 10163.