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Nanosensors for cancer detection

François Huber[a], Hans Peter Lang[a], Jiayun Zhang[a], Donata Rimoldi[b], Christoph Gerber[a]

[a] SNI, Swiss Nano Institute, Institute of Physics, University of Basel, Basel, Switzerland

[b] Ludwig Center for Cancer Research of the University of Lausanne, Épalinges VD, Switzerland

Summary

Cancer is a major burden in today's society and one of the leading causes of death in industrialised countries. Various avenues for the detection of cancer exist, most of which rely on standard methods, such as histology, ELISA, and PCR. Here we put the focus on nanomechanical biosensors derived from atomic force microscopy cantilevers. The versatility of this novel technology has been demonstrated in different applications and in some ways surpasses current technologies, such as microarray, quartz crystal microbalance and surface plasmon resonance. The technology enables label free biomarker detection without the necessity of target amplification in a total cellular background, such as BRAF mutation analysis in malignant melanoma. A unique application of the cantilever array format is the analysis of conformational dynamics of membrane proteins associated to surface stress changes. Another development is characterisation of exhaled breath which allows assessment of a patient's condition in a non-invasive manner.

Key words: *microcantilever; nanotechnology; cancer therapy; personalised healthcare; diagnostics; proteomics; genomics; metabolomics; siRNA, transcription; lab-on-a-chip*

The use of nanomechanical cantilevers goes back to the development of the atomic force microscope (AFM) [1], where a cantilever with a sharp tip at one end is utilised to image surfaces on the molecular and atomic scale. The surface of a sample is probed mechanically whereby the motion of the tip is monitored by recording the bending of the cantilever, by a laser beam reflected at the cantilever surface. In a recent development, high speed AFM has entered the time domain of chemical processes monitoring the cellular machinery at the nanoscale and millisecond resolution, for example visualisation of the movement of myosin on an actin filament in real time [2]. Complementary to imaging, biomolecular interactions on the surface of a cantilever can be studied (fig. 1). Such binding processes are analysed either by cantilever bending down to the nanoscale due to changes in surface stress based on molecular interactions on the cantilever surface (static mode, fig. 1A) [3], or exploiting changes in resonance frequencies of a vibrating cantilever due to increase in mass upon binding of biomolecules (dynamic mode, fig. 1B) [4]. The dynamic mode is similar to the operation principle of a quartz crystal microbalance [5].

In recent years, arrays of cantilevers made of silicon or silicon nitride have been batch-fabricated (fig. 1C). Various methods to measure cantilever bending or vibration have been developed, among them laser beam deflection (fig. 1D) [6], interferometry [7] and the use of piezo resistive cantilevers [8]. Functionalisation by gold-thiol chemistry allows the formation of a self-assembled monolayer (SAM) as a sensing layer. The bending of the cantilever due to ad-

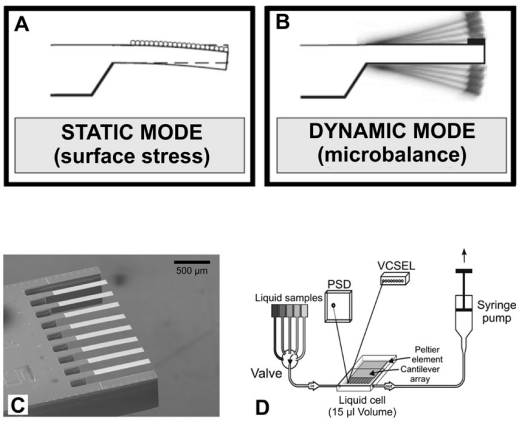

Figure 1

Two frequently used modes of cantilever sensor operation. The first one **(A)** shows the static mode where surface stress results in cantilever bending. The second mode **(B)** shows the operation in dynamic mode. Here the cantilever is actuated and vibrates at its specific resonance frequency. Upon binding of a ligand to the surface the resonance frequency changes and the adsorbed mass can be calculated from that shift. The two modes can also be used simultaneously. **(C)** Shows an electron microscope image of an array with eight 750 µm long cantilevers. **(D)** Illustrates the liquid handling system and the beam deflection read out of a cantilever setup. A total of 8 Vertical Cavity Surface Emitting Lasers (VCSEL) are used to sequentially determine cantilever deflections. The laser beam is deflected at the end of the cantilevers to a Position Sensitive Detector (PSD). Below the cantilever array is a peltier element which produces a heat pulse to test the mechanical properties of the cantilevers. Syringe pump and multi valve systems allow a constant flow of samples to the cantilever measurement chamber.

sorption and molecular interactions between the SAM and complementary molecules (fig. 2A) in solution is in the range of only a few nanometres. This was shown for the first time in DNA hybridisation experiments [9, 10], where the binding of a 12 base long oligonucleotide at a concentration of 80 nM to its complement immobilised on the cantilever surface was studied with single base-pair specificity [9]. Further investigations focused on antibody antigen interactions on cantilevers. These experiments showed sensitivity in the higher nanomolar to micromolar range, when using whole antibodies (Ab) functionalised on the surface [11]. A further improvement in antigen detection sensitivity was achieved by using oriented single chain antibody fragments of the variable domain (scFv), which increased the sensitivity about 500 times to low nanomolar concentrations due to their smaller size and uniform orientation on the cantilever surface [12]. Numerous applications lie in the study of structural changes in proteins. This domain is particularly suited for static mode measurements. Structural changes are often needed for protein activity, for example to perform certain functions like enzymatic conversion or binding of ligands. These changes can increase the surface stress in a monolayer producing bending of a cantilever [13]. A combination of DNA and protein detection was used to investigate the binding of transcription factors [14]. Here a thiolated double stranded hairpin structured oligonucleotide (dsOligo) was used to functionalise the gold surface of a cantilever. The dsOligos contained binding sites specific for the transcription factor NF-κB or SP1. Both transcription factors are important in mediating various cellular responses and thus can play a role both in cancer development [15] and in tumour response to treatment [16]. Cantilever bending occurred when a transcription factor recognised and bound to its target sequence on the cantilever. The dynamic mode can be applied to study virus adsorption [17]. Viruses can bind to surface immobilised ligands, resulting in a change of resonance frequency, indicating a change of mass on the cantilever surface.

An important step towards simplifying cancer diagnostics with nanomechanical cantilevers was achieved recently by analysing single point mutations in total RNA from melanoma cells [18] (fig. 2B). Malignant melanoma, the deadliest form of skin cancer, is characterised by a prevalent single point mutation in the *BRAF* gene occurring in 50% of all cases. Highly specific drugs like vemurafenib are now available that target this mutation, and therefore patients have to be screened to determine their treatment eligibility. This is important as these new drugs can cause severe side effects. *BRAF* is one of three *RAF* genes (rapidly accelerated fibrosarcoma A, B and C) encoding cytoplasmic protein serine/threonine kinases belonging to the mitogen-activated protein kinase (MAPK) signal transduction cascade, a pathway controlling various cellular processes such as proliferation, migration and survival. There are different methods to detect the mutation, among them is the long established procedure of polymerase chain reaction (PCR) amplification coupled with Sanger sequencing of the product. The standard test currently used to analyse patients' biopsies before initiation of vemurafenib treatment relies on a real-time PCR-based assay, the so called COBAS test [19].

Both approaches rely on amplification and labelling of RNA or DNA extracted from melanoma cells. This is a

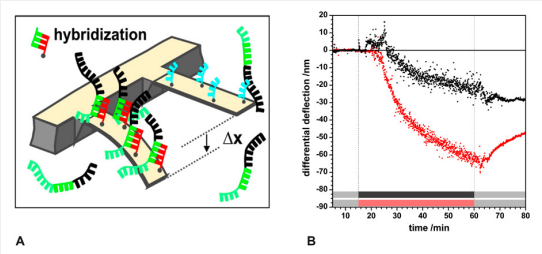

Figure 2

(A) The example of DNA hybridisation to immobilised oligonucleotides illustrates the molecular crowding, which creates a surface stress resulting in cantilever bending. A differential deflection (Δx) can be measured with the help of a reference cantilever. **(B)** Measurement of total RNA from a BRAF mutated melanoma cell line (red) and from a cell line (black) that does not contain the BRAF mutation. Injection is indicated with a red and black bar at the bottom of the graph. The measurement device is washed with buffer before and after injection. The two cell lines can be clearly distinguished.

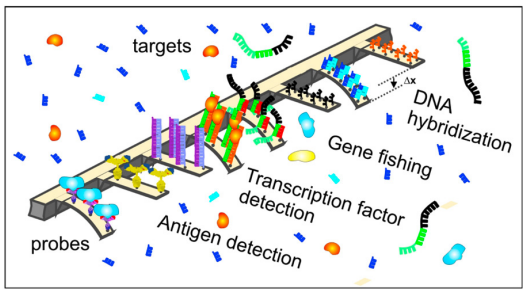

Figure 3

This figure illustrates a future application for cantilever arrays, where cantilevers in a single array are functionalised with a variety of biomolecules, ranging from antibodies to double strand DNA to single strand DNA. In such a configuration, it will be possible to investigate multiple markers with one cantilever array.

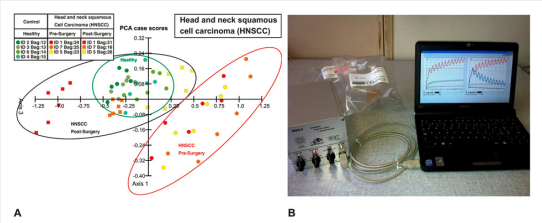

Figure 4

(A) Principal Component Analysis plot showing three distinct clusters (indicated with ellipses) that represent healthy control persons, head and neck squamous cell carcinoma (HNSCC) patients before surgery and HNSCC patients after surgery, i.e. after removal of the tumour by surgery. The points of the HNSCC patients after surgery are at a similar location in the PCA plot as those from the healthy persons and differ clearly from the points of the HNSCC patients before surgery, indicating that the removal of the tumour has been successful. **(B)** Portable USB-powered setup for mobile characterisation of patients' breath samples using polymer-coated membrane surface stress sensors. From left to right: Measurement system containing chamber with piezo membrane sensor array and pumps for aspiration of sample; breath sample bags; netbook with operating and analysis software.

potential shortcoming because every additional modification, by labelling or amplification, may disturb the original content of the sample and consume additional time. Contrary to these procedures, total RNA samples used for cantilevers do not have to be labelled and amplification is not necessary, leaving the original sample undisturbed. In Huber et al. [18] different cantilevers were coated with thiol DNA oligonucleotides representing the mutated gene, a reference sequence and the wild type gene. The wild type gene serves as an additional reference as the melanoma cells express the mutant as well as the wild type gene albeit usually at a lower level. Thiol oligonucleotides covering the cantilever are able to form a heteroduplex with the corresponding RNA in the sample and thereby identify the BRAF mutation. The high sensitivity of cantilevers always makes it necessary to include a reference cantilever to avoid false positive responses. This can also be accomplished by differentially coating the upper and the lower side of cantilevers [20]. The method described in Huber et al. [18] was capable of distinguishing BRAF wild type cells from BRAF mutated cells. In further experiments we applied total RNA from different melanoma cell lines to evaluate the robustness of the method. Some of these samples contained the prevalent BRAF mutation while others did not carry the mutation or carried a different mutation in the same gene. We also applied the method to investigate interferon treatment by analysing mRNA from interferon treated melanoma cells [21] in collaboration with U. Certa's group of the Roche Centre for Medical Genomics. Interferon has powerful anti-proliferative properties and is used in treatment of viral diseases and cancer. However, interferon treatment also has severe side effects, which together with the occurrence of resistance reduces its usefulness in cancer treatment [22]. A better insight in the molecular mechanisms behind these effects could result in a better understanding of resistance to drugs and the development of improved cancer treatments. For this purpose, they have applied DNA microarrays with a set of probes to more than 11,000 human transcripts to study the expression of interferon inducible genes in a sensitive and resistant melanoma cell line. It was found that only few genes were either up or down regulated and could be used as potential markers to indicate tumour cell sensitivity or resistance to interferon. The approach relied on the amplification and labelling of the RNA extracted from the melanoma cells. As discussed previously this can be a potential shortcoming. Samples used for cantilevers do not have to be modified with flourophores for detection and in some cases, PCR amplification is also not necessary, leaving the sample in its original state. In Zhang et al. [21] different cantilevers were coated with thiol oligonucleotides representing one of the interferon induced genes, a so-called housekeeping gene and a reference sequence. The housekeeping genes serve as a positive control as this type of gene is necessary for the survival of all cells and therefore are usually expressed at constant levels. Zhang et al. [21] clearly showed the induction of expression of an interferon inducible gene, without sample labelling, though some amplification was still necessary due to low levels of expression. In further experiments with total cellular RNA the housekeeping gene, Aldolase A was detected without amplification, labelling or modification of the cellular sample at all. The latest development described in Huber et al. [18] above, shows that amplification is not necessary either.

The sequencing of the entire human genome has revealed that about 22,000 genes encode proteins. This corresponds to about 1.5% of the whole genome. As recently discovered, a small part of the 98.5% of the non-coding DNA is involved in regulation of mRNA translation through the expression of non-coding RNA sequences, including small interfering RNAs (siRNAs). These species of RNA are able to interfere with mRNA, thereby suppressing the translation or enhancing the degradation process of specific mRNAs. The siRNAs are short RNA molecules of approximately 20 bases and should therefore be amenable to analysis with cantilevers. Specific sets of siRNA are down or up-regulated in cancer cells, and the use of siRNA in cancer therapy has been recently suggested [23]. Cantilever array technology could provide a possible way to monitor the efficiency of such therapies.

From the many thousand genes analysed in microarray-based systems, a limited set is often sufficient to define a particular subtype of cancer or is used to guide therapeutic decisions. Cantilever arrays can provide a snapshot of clinically relevant gene expression. For the future, we foresee cantilever arrays functionalised with appropriate sets of cancer-relevant genes to quickly and reliably analyse patient samples. While the cantilever arrays highlighted in this review are one dimensional and contain only 8 cantilevers, in some developments two dimensional arrays of 8 × 8 cantilevers [24], lab-on-a-disc systems with many 100 cantilevers [25] are used. Current production technologies can produce larger arrays of 100 × 100 cantilevers [26], although so far these are used for data storage purposes and not for biosensors.

Cancer is a complex disease and while analysing differences in expression levels is important, these data alone are not enough to follow the effectiveness of a therapy for example, and the analysis of other cellular components and extracellular markers is mandatory. The technology we describe in this review is able to monitor cellular antigens (with the help of immobilised antibodies), transcription factors (using as bait relevant DNA consensus sequences) or RNAs (using specific complementary sequences). A study conducted by Wu et al. [27] using antibody functionalised cantilevers has shown that prostate specific antigen (PSA) can be detected at clinically relevant concentrations. PSA is an important marker in diagnosing prostate cancer. This example and others described above show that a combination of DNA, RNA and antibodies on a single array would be desirable (fig. 3). This way the genome and the proteome could be analysed with a single cantilever array.

The examples mentioned above show that nanomechanical sensors are well in the sensitivity range for clinical applications, such as the detection of PSA which is in the range of 0.2 ng/ml to 60 µg/ml [27], the identification of other medically relevant biomarkers [11, 12] down to 1 nM and the test for specific mutations in cancers [18] down to 10 pM. Specifically the last example gives us an indication that biopsies from patients can be investigated, for example the distinction of the BRAFV600E mutation from wild type BRAF in melanoma biopsies. Distinguishing BRAFV600E

from BRAF also shows the high specificity of our technology, because the BRAFV600E differs in a single base (a T to A transversion at position 1799 of the gene) and we accomplish this in total RNA extracted from cells.

Cantilever arrays can be applied not only to liquid samples but also to gaseous samples, such as breath samples of cancer patients. In this application a non-invasive characterisation of breath samples can be achieved [28], through the detection of volatile metabolites. Here cantilevers are coated with different polymers that upon exposure to gaseous mixtures are able to absorb volatile metabolites in a characteristic way. This process will lead to polymer swelling and thereby produce a surface stress creating a distinct response pattern for each sensor in the array. Physicians have known for centuries that diseases indeed leave traces of specific volatile organic compounds (VOC) in a patient's breath [29]. Analysing these components with cantilever array technology can allow the identification of various cancers, for example head and neck cancer in a non-invasive way (fig. 4A). Novel piezo-resistive membrane-type sensor arrays for cancer diagnosis have led to a smaller compact system that is portable and powered by a laptop computer (fig. 4B). Indeed a clinical study conducted recently with the membrane type sensors was very promising in distinguishing healthy, sick and treated individuals [30].

The results obtained through the cantilever sensor technology are robust in signal response, but can sometimes vary by a factor of 2 due to alignment of the lasers on the cantilevers. Along that line we think that major improvements will come from progress in device and software development, especially for easier handling of the cantilever arrays and the device. Eventually we expect the technology to be cheaper than current methods such as the COBAS test, once volume production of devices and arrays increases. The next important step for medical applications of nanomechanical cantilever biosensors lies in further miniaturisation of the technology. In particular the liquid handling system can be reduced down to nanolitre or picolitre volumes resulting in so-called microfluidic networks. While reducing the dimensions of the cantilevers is one possibility to increase sensitivity, especially in dynamic mode, further improvements can be achieved by either increasing the density of the probe molecules on the cantilever surface or bringing the interaction sites closer to the cantilever surface, where the surface stress is generated. Further miniaturisation of the whole cantilever system would allow the development of small portable devices and the use of very small sample volumes with the possibility of using cantilever arrays in miniaturised total analysis systems (μTAS) [31] or lab on a chip. We envision a small and compact biosensor system based on cantilever technology which is capable of analysing a patient's health at different levels by interrogating its genome, proteome or metabolome. Furthermore, such devices may be used to monitor the efficacy of a given cancer treatments in real time, so that appropriate changes in treatment regimen can be rapidly implemented. All these developments could contribute to personalised healthcare in cancer treatment.

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Correspondence: *François Huber, PhD, SNI, Swiss Nano Institute, Institute of Physics, University of Basel, Klingelbergstrasse 82, CH-4056 Basel, Switzerland, Francois.Huber[at]unibas.ch*

References

1 Binnig G, Quate CF, Gerber C. Atomic Force Microscope. Phys Rev Lett. 1986;56(9):930–3.

2 Kodera N, Yamamoto D, Ishikawa R, Ando T. Video imaging of walking myosin V by high-speed atomic force microscopy. Nature. 2010;468(7320):72–7.

3 Thundat T, Warmack FLJ, Chen GY, Allison DP. Thermal and ambient-induced deflections of scanning force microscope cantilevers. Appl Phys Lett. 1994;64(21):2894–6.

4 Thundat T, Chen GY, Wannack RJ, Allison DP, Wachter EA. Vapor detection using resonating microcantilevers. Anal Chem. 1995;67(3):519–21.

5 Marx KA. Quartz crystal microbalance: a useful tool for studying thin polymer films and complex biomolecular systems at the solution-surface interface. Biomacromolecules. 2003;4(5):1099–120.

6 Meyer G, Amer NM. Novel optical approach to atomic force microscopy. Appl Phys Lett. 1988;53(12):1045–7.

7 Helm M, Servant JJ, Saurenbach F, Berger R. Read-out of micromechanical cantilever sensors by phase shifting interferometry. Appl Phys Lett. 2005:87(6):064101.

8 Datskos PG, Oden PI, Thundat T, Wachter EA, Warmack RJ, Hunter SR. Remote infrared radiation detection using piezoresistive microcantilevers. Appl Phys Lett. 1996;69(20):2986–8.

9 Fritz J, Baller MK, Lang HP, Rothuizen H, Vettiger P, Meyer E, et al. Translating biomolecular recognition into nanomechanics. Science. 2000;288(5464):316–8.

10 McKendry R, Zhang J, Arntz Y, Strunz T, Hegner M, Lang HP, et al. Multiple label-free biodetection and quantitative DNA-binding assays on a nanomechanical cantilever array. Proc Natl Acad Sci USA. 2002;99(15):9783–8.

11 Arntz Y, Seelig JD, Lang HP, Zhang J, Hunziker P, Ramseyer JP, et al. Label-free protein assay based on a nanomechanical cantilever array. Nanotechnology. 2003;14(1):86–90.

12 Backmann N, Zahnd C, Huber F, Bietsch A, Plückthun A, Lang HP, et al. A label-free immunosensor array using single-chain antibody fragments. Proc Natl Acad Sci USA. 2005;102(41):14587–92.

13 Braun T, Backmann N, Vögtli M, Bietsch A, Engel A, Lang HP, et al. Conformational change of bacteriorhodopsin quantitatively monitored by microcantilever sensors. Biophys J. 2006;90(8):2970–7.

14 Huber F, Hegner M, Gerber C, Güntherodt HJ, Lang HP. Label free analysis of transcription factors using microcantilever arrays. Biosens Bioelectron. 2006;21(8):1599–605.

15 Xie K, Wei D, Huang S. Transcriptional anti-angiogenesis therapy of human pancreatic cancer. Cytokine Growth Factor Rev. 2006;17(3):147–56.

16 Criswell T, Leskov K, Miyamoto M, Luo G, Boothman DA. Transcription factors activated in mammalian cells after clinically relevant doses of ionizing radiation. Oncogene. 2003;22(37):5813–27.

17 Braun T, Ghatkesar MK, Backmann N, Grange W, Boulanger P, Letellier L, et al. Quantitative, time-resolved measurement of membrane protein-ligand interactions using microcantilever array sensors. Nat Nanotech. 2009;4(3):179–85.

18 Huber F, Lang HP, Backmann N, Rimoldi D, Gerber C. Direct detection of a BRAF mutation in total RNA from melanoma cells using cantilever arrays. Nat Nanotech. 2013;8(2):125–9.

19 Halait H, DeMartin K, Shah S, Soviero S, Langland R, Cheng S, et al. Analytical Performance of a Real-time PCR-based Assay for V600 Mutations in the BRAF Gene, Used as the Companion Diagnostic Test for the Novel BRAF Inhibitor Vemurafenib in Metastatic Melanoma. Diagn Mol Pathol. 2012;21(1):1–8.

20 Rasmussen PA, Grigorov AV, Boisen A. Double sided surface stress cantilever sensor. J Micromech Microeng. 2005;15(5):1088–91.

21 Zhang J, Lang HP, Huber F, Bietsch A, Grange W, Certa U, et al. Rapid and label-free nanomechanical detection of biomarker transcripts in human RNA. Nat Nanotech. 2006;1(3):214–20.

22 Certa U, Wilhelm-Seiler M, Foser S, Broger C, Neeb M. Expression modes of interferon-α inducible genes in sensitive and resistant human melanoma cells stimulated with regular and pegylated interferon-α. Gene. 2003;315:79–86.

23 Wall NR, Shi Y. Small RNA: can RNA interference be exploited for therapy? Lancet. 2003;362(9393):1401–3.

24 Raorane D, Lim SH, Majumdar A. Nanomechanical Assay to Investigate the Selectivity of Binding Interactions between Volatile Benzene Derivatives. Nano Lett. 2008;8(8):2229–35.

25 Bosco FG, Hwu ET, Chen CH, Keller S, Bache M, Jakobsen MH. High throughput label-free platform for statistical bio-molecular sensing. Lab Chip. 2011;11(14): 2411–16.

26 Kim YS, Lee CS, Jin WH, Jang S, Nam HJ, Bu JU. 100x100 thermo-piezoelectric cantilever array for SPM nano-data-storage application. Sens Mat. 2005;17(2):57–63.

27 Wu G, Datar RH, Hansen KM, Thundat T, Cote RJ, Majumdar A. Bioassay of prostate-specific antigen (PSA) using microcantilevers. Nat Biotechnol. 2001;19(9):856–60.

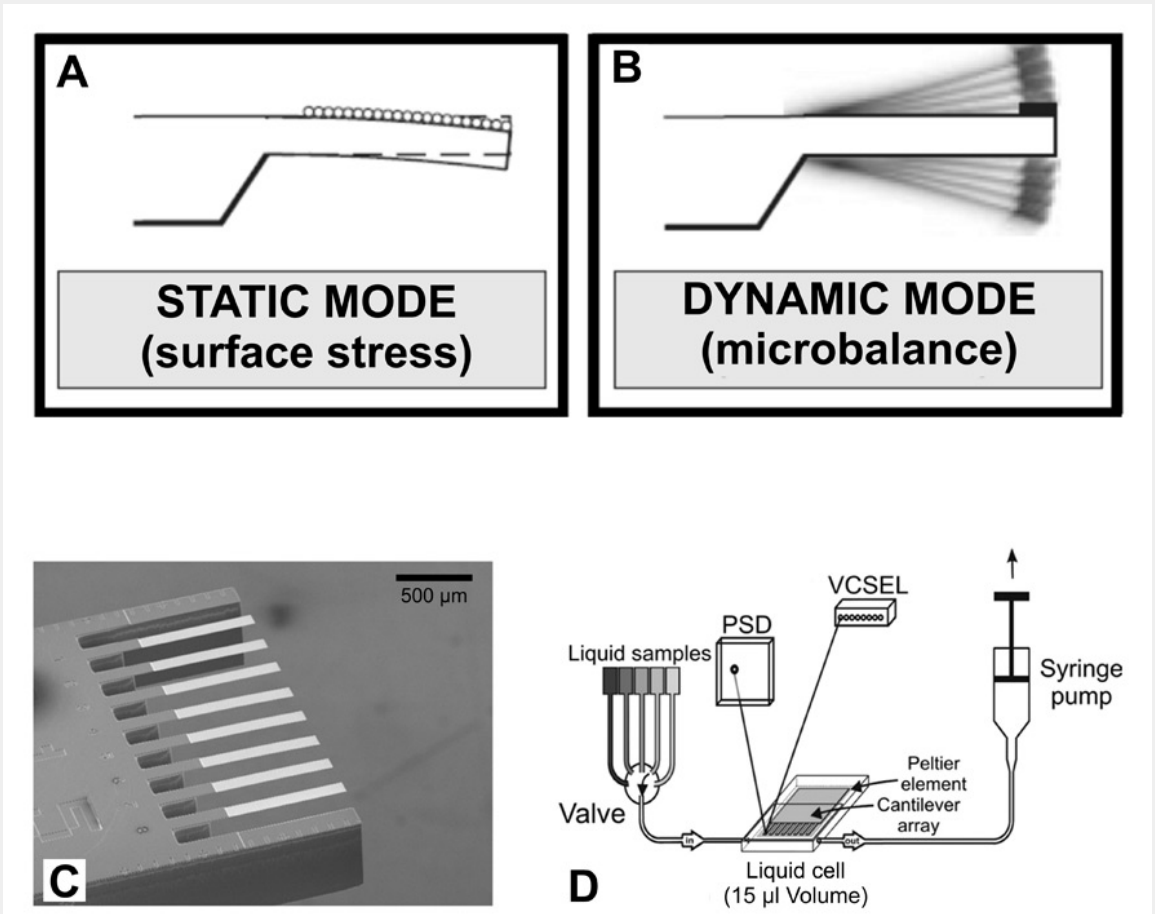
28 Lang HP, Hegner M, Meyer E, Gerber C. Nanomechanics from atomic resolution to molecular recognition based on atomic force microscopy technology. Nanotechnology. 2002;13(5):R29–R36.

29 Phillips M, Gleeson K, Hughes JMB, Greenberg J, Cataneo RN, Baker L, et al. Volatile organic compounds in breath as markers of lung cancer: a cross-sectional study. Lancet. 1999;353(9168):1930–3.

30 Loizeau F, Lang HP, Akiyama T, Gautsch S, Vettiger P, Tonin A, et al. Piezoresistive membrane-type surface stress sensor arranged in arrays for cancer diagnosis through breath analysis. Proceedings of the IEEE International Conference on Micro Electro Mechanical Systems (MEMS); 2013 Jan 20–24; Taipei, TWN. Amsterdam: Elsevier B.V. 2013. p. 621–4.

31 Manz A, Graber N, Widmer HM: Miniaturized total chemical analysis systems: a novel concept for chemical sensing. Sens Actuators B Chem. 1990;1(1–6):244–8.

Figures (large format)



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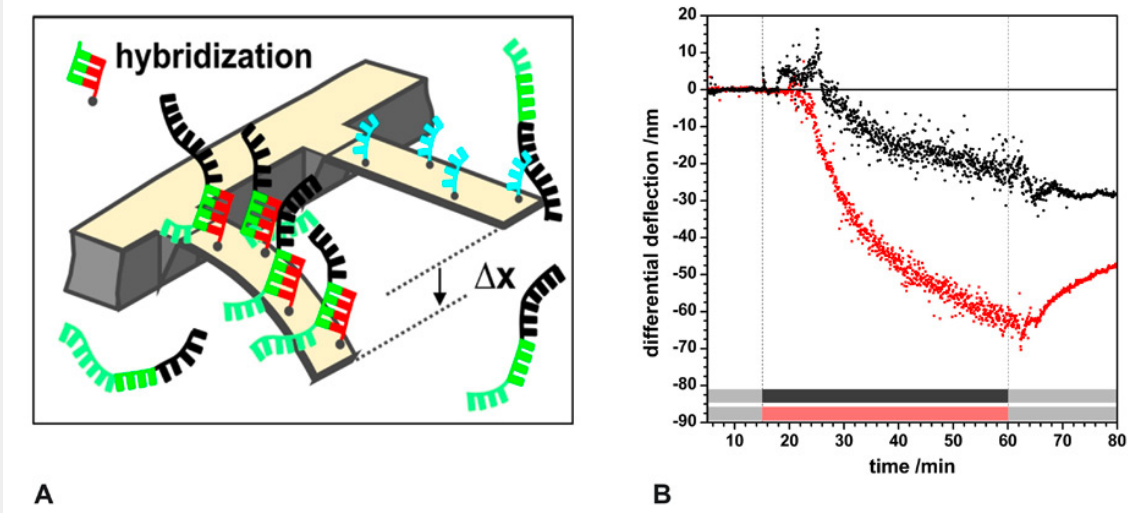

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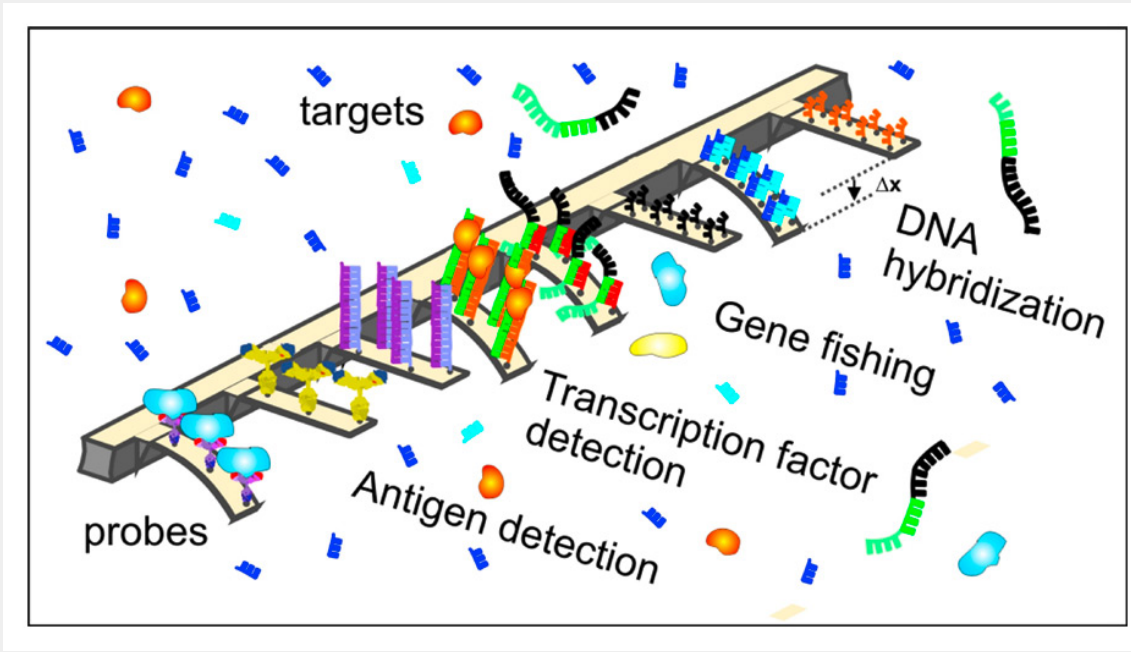

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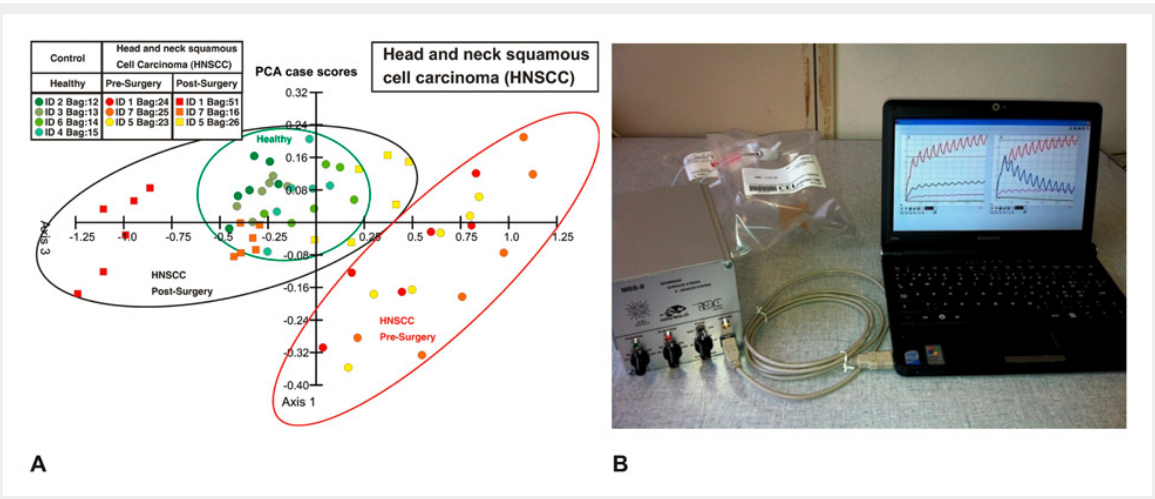

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