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PAPER

High throughput label-free platform for statistical bio-molecular sensing†

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Sensors are crucial in many daily operations including security, environmental control, human diagnostics and patient monitoring. Screening and online monitoring require reliable and high-throughput sensing. We report on the demonstration of a high-throughput label-free sensor platform utilizing cantilever based sensors. These sensors have often been acclaimed to facilitate highly parallelized operation. Unfortunately, so far no concept has been presented which offers large datasets as well as easy liquid sample handling. We use optics and mechanics from a DVD player to handle liquid samples and to read-out cantilever deflection and resonant frequency. Also, surface roughness is measured. When combined with cantilever deflection the roughness is discovered to hold valuable additional information on specific and unspecific binding events. In a few minutes, 30 liquid samples can be analyzed in parallel, each by 24 cantilever-based sensors. The approach was used to detect the binding of streptavidin and antibodies.

1. Introduction

Micrometre and even nanometre sized cantilevers have been studied since the mid-1990s and used for label free molecular recognition.[1–7] There, the cantilever is typically functionalized with probe molecules designed to specifically bind certain target molecules in solution. The specific binding of target molecules causes the cantilever to deflect due to a change in surface stress. Alternatively, the mass change of the cantilever can be monitored by measuring the resonant frequency change of the cantilever which is inversely proportional to the added mass. Cantilevers are unique probes for studying, for example, small molecule drug binding interactions,[8] membrane protein–ligand interactions[9] and DNA hybridization.[10] Often the microscopic size of the cantilevers is highlighted as a virtue for parallelization. Parallelization and easy sample handling is needed for high throughput screening of thousands of liquid samples per hour. This has to date not been solved and a solution is crucial for a potential commercial breakthrough of the sensor technology.

2. Methods for parallel cantilever sensing

Today, the prevalent method of monitoring vibrational amplitudes and cantilever deflections is based on the optical leverage technique[11] widely used in atomic force microscopy.[12] Such systems are typically bulky because of the requirement for a long optical path. Also, the focusing of the laser spot on the cantilever and the alignment of the laser beam on the optical detector are tedious and time consuming. For research purposes several optical leverage based read-out systems have been developed.[13–15]

Alternatively, a CCD camera has been used for monitoring cantilever deflection and hereby large 2-dimensional cantilever arrays can be read simultaneously.[16] However, all cantilevers have to be in the same focal plane which is extremely difficult to achieve in practice and both techniques only apply to micrometre sized cantilevers. In optical leverage the laser spot size is typically 20 μm or above and in the CCD system the amount of reflected light is too low for smaller devices. Integrated read-out has been suggested by several groups. For example, cantilevers with piezoresistive,[17,18] piezoelectric[19,20] and MOSFET-based[21,22] read-outs have been developed and applied for molecular recognition. Generally, these cantilevers have to be carefully insulated in order to be operated in liquid and the devices require significantly more packaging due to electrical interconnections.

Sensing is normally performed on one or maybe two cantilevers at a time (one for reference) since measurements are rather elaborate and time consuming. Typically, the cantilevers are placed in small polymer or ceramic chambers and different liquids are introduced using *i.e.* syringe pumps. The pumps are a potential noise source and the liquid handling is performed in a serial manner and is slow. Because of primarily the instrumentation, few papers on cantilever-based sensing present statistically analyzed datasets.

3. A high throughput platform

We report on a DVD based sensor platform that significantly reduces the aforementioned obstacles and challenges in

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cantilever based sensing and liquid handling. The concept is highly scalable and we can in this initial design analyze 30 different liquid samples in parallel. Also, the monitoring of three physical parameters (deflection, resonant frequency and optical roughness) increases the reliability of the data. A DVD shaped disk is used to mount up to 90 cantilever chips, each with 8 cantilevers, in a radial symmetry, see Fig. 1a. In this work, silicon cantilevers with a length of 500 μm, a width of 100 μm and a thickness of 1 μm have been used.[23] All cantilevers are coated on one side with a 20 nm thick gold layer. The gold layer ensures a good reflectivity of the cantilever surface and can be used for functionalization when using thiol-based chemistry. The cantilever chips are clicked into individual reservoirs.[24] Approximately 1 mm below the disk a DVD-ROM optical pickup head (PUH) provides the read-out system. The disk is spun and cantilevers are illuminated by the DVD laser with a wavelength of 650 nm and a spot diameter of only 0.56 μm (full width half maximum).

A reflective aluminium pattern on the disk surface ensures that the DVD-ROM PUH maintains the focus distance. The laser scans from the bottom, passes through the glass substrate and focuses on the cantilever surface (Fig. 1b). The deflection profiles are measured using the astigmatic detection mechanism normally used for autofocusing[25] (see ESI†). The PUH can measure the cantilever deflection with sub-nanometric resolution in Z direction. We have measured cantilever deflections at rotating velocities up to 120 rpm, which equals approximately 500 cantilevers per second. Typical sampling rate corresponds to around 1000 measurement points across the width of each cantilever. We thus obtain a profile where data points are acquired every 100 nm along the width of the cantilever. By combining cantilever scans at sequential radial positions it is possible to construct a 3D image of the cantilever surfaces, see Fig. 1c. In our work, the measured surface roughness is used to evaluate the distribution of biomolecules on the cantilever surface. When inhomogeneous binding of material occurs, the optical properties (refractivity and reflectivity) change, resulting in a "rough" surface.

Finally, the system can measure changes in the resonant frequency of the cantilever using the thermal noise peaks of the cantilevers.[26] In these measurements the laser has to dwell for approximately 1 second at the cantilever apex (Fig. 1d).

The DVD disc format has in the past 10 years been widely used for liquid handling. The centrifugal forces generated by spinning the disc can be used to move liquid from the center of the disc and towards the edge.[27,28] We have fabricated microfluidic channels connecting the chip reservoirs. Each liquid sample is delivered to

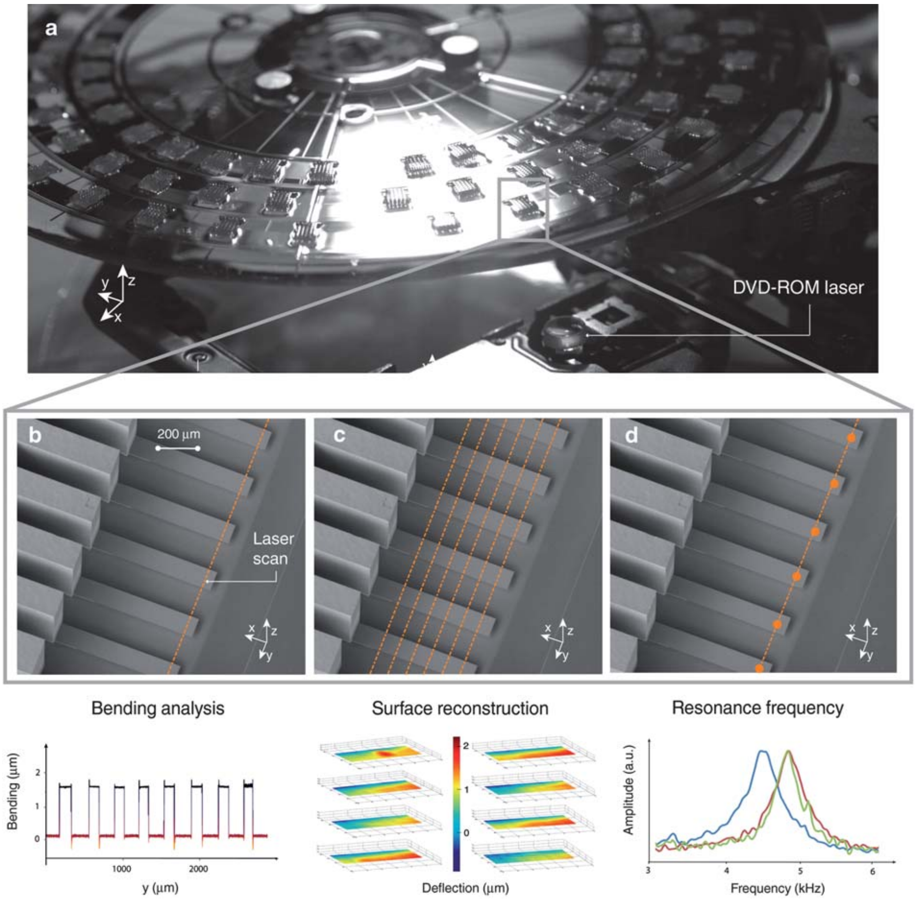

Fig. 1 First generation high-throughput nanomechanical sensor platform. (a) Photograph of a DVD platform with integrated cantilever chips. The disc is fabricated in glass and in the polymer SU-8. (b—d) Scanning Electron Microscope image of gold-coated silicon cantilevers with dimensions 100 μm × 500 μm × 1 μm. The laser movement and the resulting data are shown for three different data acquisition modes: (b) deflection, (c) surface roughness and (d) resonant frequency.

three reservoirs placed in series, starting at the center and ending at the outer rim of the disc.

Raw cantilever deflection signals acquired during one revolution of the disc are shown in Fig. 2a. The plot is composed of around 1 000 000 data points. Each distinguishable peak represents a chip with 8 cantilevers. Typical experiments consist of 30–50 revolutions, resulting in up to 50 million measurement points. Zooming in on Fig. 2a we can extract the individual cantilever profiles, as seen in Fig. 2b.

Before sensing experiments are performed, each cantilever is fully characterized by at least 10 revolutions of the disk. The variance of the measurements is used to evaluate the reliability of the data. After data processing, it is possible to obtain a detailed statistical analysis of the initial conditions of the cantilevers in air. The histogram in Fig. 2c shows the distribution of initial cantilever bending from 30 chips (240 cantilevers) measured over 10 revolutions. The average bending is 0.49 μm, with a standard deviation of 0.43 μm.

As a proof of concept we have performed liquid phase reactions which are monitored in dry phase. That is, the cantilever characteristics are measured in air before and after exposure to liquid samples. A similar approach has previously been used for *i.e.* bacteria detection.[29] By doing so the complexity of the measurements is reduced. For example, resonant frequency detection would, due to damping, be challenging to perform in the liquid.

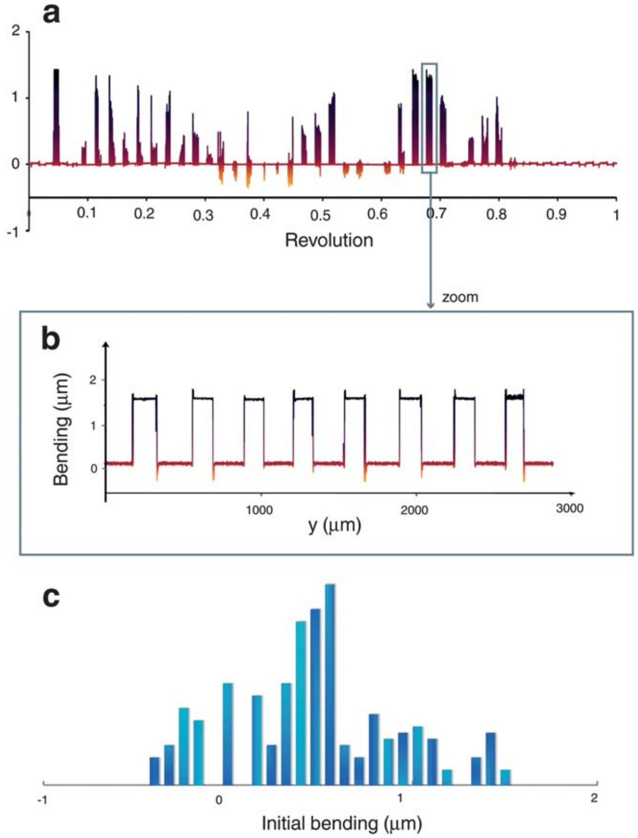

Fig. 2 Demonstration of parallel data acquisition. (a) Raw data from one revolution of the DVD. Each peak corresponds to one cantilever chip. (b) The obtained profiles from a single cantilever chip. (c) Distribution of the measured initial bending of 240 cantilevers in total.

4. Molecular recognition

For a first demonstration of biomolecular binding, 8 cantilevers were functionalized with thiolated biotin and 8 untreated cantilevers were used for reference measurements. The chips were inserted into reservoirs in the DVD platform and a buffer solution containing streptavidin was injected into the reservoirs.[30] After 10 min of exposure to streptavidin, all cantilevers were washed in buffer and subsequently water. The water was left to evaporate. The cantilever responses were measured continuously before and after incubation with streptavidin. During incubation and washing the disc rotation was halted and data were not acquired. Fig. 3a shows the averaged 3D surface reconstruction of the gold side of 8 untreated cantilevers, measured before the injection of streptavidin. These surfaces have a roughness of a few nm, indicating that the gold layer is clean. The initial end point deflection of the untreated cantilevers is around 5 μm. After injection of streptavidin and washing the same cantilevers show a high increase in surface roughness, suggesting that an inhomogeneous layer has formed (Fig. 3b). This indicates that streptavidin has bound unspecifically to the untreated cantilever surfaces as illustrated in the schematics in Fig. 3b.

The cantilevers functionalized with biotin are initially bent 5–6 μm at the cantilever apex and the surface appears optically smooth, see Fig. 3c. This reflects that the biotin functionalization has created a monolayer on the gold surface of the cantilevers. After the biotin–streptavidin binding has occurred, the observed change in cantilever bending is approximately 3 μm and the roughness of the surface appears unchanged, indicating that streptavidin has been uniformly bound to the biotin layer, Fig. 3d.

Fig. 3e and f compare data from the untreated and the biotin functionalized cantilevers. Each data point corresponds to the averaged value from 8 cantilevers. We notice that after the incubation with streptavidin the bending of the untreated cantilevers decreases, reaching an asymptotic value after around 15 disc revolutions (corresponding to approximately 5 minutes), see Fig. 3e.

At this stage the water has fully evaporated from the reservoir and stable measurement conditions can be obtained. Similar behavior (but in the opposite direction) is observed for the biotin functionalized cantilevers. These biotin functionalized cantilevers show an averaged change in deflection which is approximately 2 μm larger than for the untreated reference cantilevers when the measurements have stabilized. The averaged change in surface roughness (Fig. 3f) is significant for the untreated cantilevers compared with the functionalized ones, suggesting that an irregular streptavidin layer is formed on the untreated cantilever whereas a uniform layer, as expected, is formed on the biotin-coated surface. Thus, the specific binding of streptavidin results in significant differential changes (biotin functionalized minus untreated cantilever) in cantilever deflection and surface roughness.

Similar experiments have been performed for detection of the pesticide derivative 2,6-dichlorobenzamide (BAM).[31] The used protocol has been developed for a competitive assay which implies that the sensing cantilevers are initially coated with a layer of BAM.[32] Two chips have been prepared for the measurements, each containing 2 cantilevers functionalized with

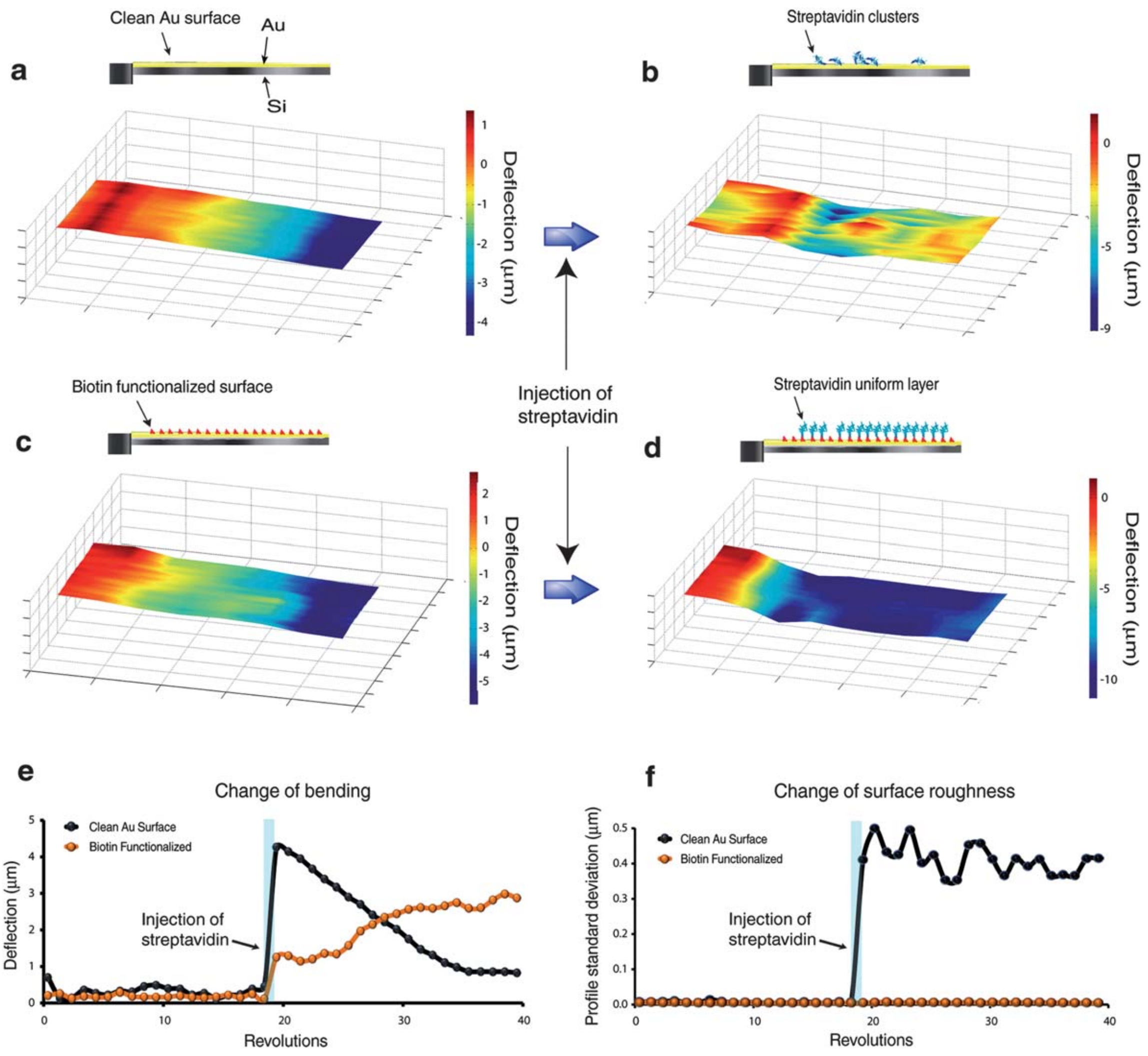

Fig. 3 Detection of biotin–streptavidin binding. (a) Surface reconstruction of clean cantilever. (b) The same cantilever after exposure to streptavidin solution showing increased roughness. (c) Surface reconstruction of biotin functionalized cantilever and (d) of the same cantilever after reaction with streptavidin. (e and f) Averaged change in cantilever bending and surface roughness for an untreated surface (average value of 8 cantilevers) and a biotin functionalized surface (average value of 8 cantilevers). At revolution 19 the cantilevers are incubated for 10 minutes in streptavidin.

BAM molecules in ovalbumin matrix, 2 cantilevers with an ovalbumin blocking layer and 4 untreated cantilevers. The initial bending of the cantilevers is measured as above. Specific antibodies against BAM are injected into the cantilever reservoirs followed by a rinse in water and subsequent water evaporation. Fig. 4a shows the induced averaged bending of the differently functionalized cantilevers. The BAM-functionalized cantilevers deflect approximately 10 µm compared with 3–5 µm for the untreated and ovalbumin coated cantilevers. Probably, the antibodies bind strongly to the BAM functionalized surfaces causing a large change in surface stress whereas they bind unspecifically to the other cantilevers, illustrated in Fig. 4b. After the introduction of antibodies, the analyzed profiles reveal that the untreated cantilevers become significantly rougher, while the change in optical roughness for BAM and ovalbumin coated surfaces is smaller, due to their already rough functionalization coatings. It is however noticeable that ovalbumin-blocked cantilever roughness increases 3 times more than the BAM one, whose surface has presented similar initial optical characteristics, see Fig. 4c. We believe that this is once again an indication that the specific binding results in more ordered uniform layers whereas the unspecific binding results in a random surface arrangement and thus in a rougher surface. We conclude that the specific binding of BAM antibodies is detectable due to large differential signals in both deflection and surface roughness.

In the BAM experiments we have also tested the capability of the system to measure changes in the resonant frequency. Fig. 4d shows the change in resonant frequency for the 16 cantilevers after reaction with antibodies has taken place and the washing solution has evaporated. The BAM functionalized cantilevers have the highest change in resonant frequency (approximately 10%). Compared to that, minor changes are observed for the ovalbumin blocked and the untreated cantilevers (1–2%), which can be attributed to unspecific binding of antibodies as well as solidification of salt present in the buffer solution. The observed frequency changes in the specifically treated cantilevers are positive, which is probably a result of changes in surface stress or of a combination between surface effects and added mass.[33]

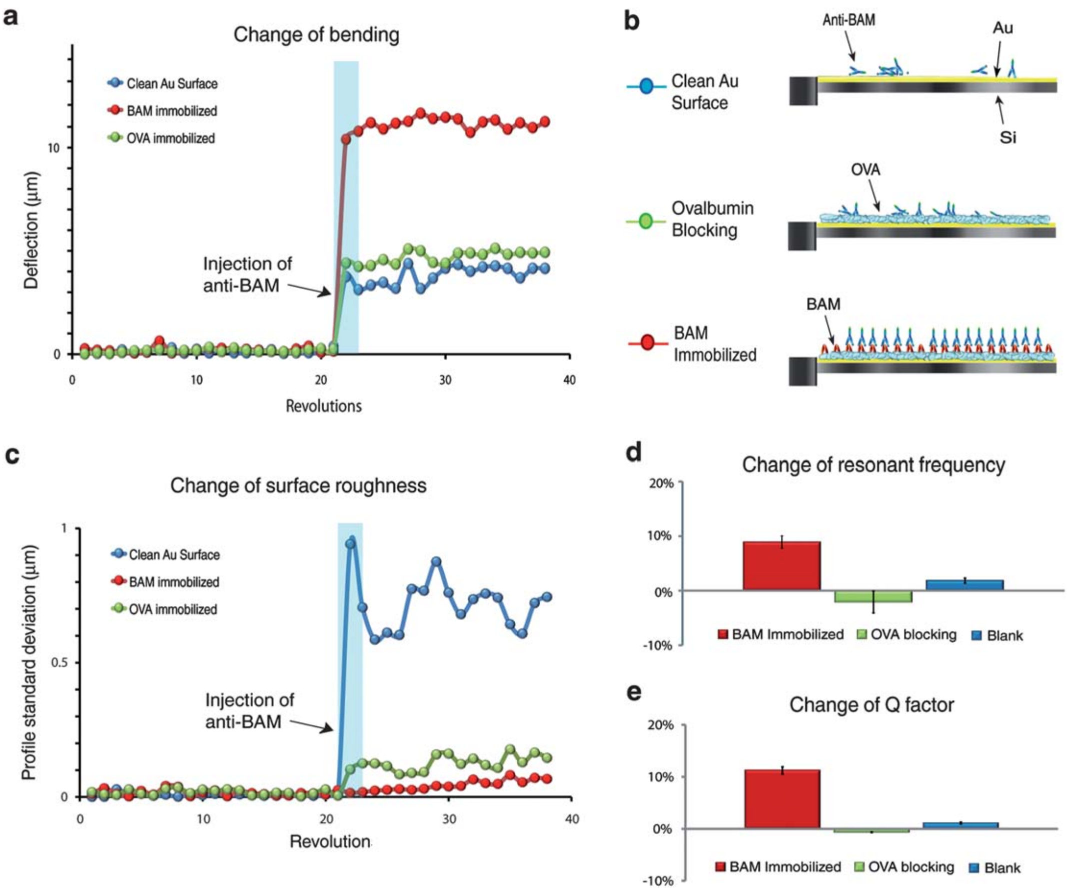

Fig. 4 Detection of BAM antibodies. (a) Averaged changes in cantilever deflections when exposed to BAM antibodies. All data points represent averaged values from either 4 (ovalbumin and BAM coated) or 8 (untreated gold-coated) cantilevers. (b) Graphical representation of the differently coated cantilevers. (c) Averaged changes in surface roughness after exposure to BAM antibodies. (d and e) Measured averaged changes in resonant frequency and Q-factor. The significant change for the BAM coated cantilevers indicates binding of the BAM antibodies. At revolution 21 the cantilevers are incubated for 10 minutes in the anti-BAM solution.

The corresponding Q-factors of the cantilevers extracted from the resonant curves (Fig. 4e) generally follow the changes in resonant frequency, suggesting that an increase in the stiffness of the cantilevers due to the addition of the surface layer has occurred. Uniformly added mass on the cantilevers could have increased the stored energy of the resonators to produce the increase of the Q-factor, however the theoretical relationship between the surface stress change and the stiffness of cantilever beam is still under debate.[34]

Conclusion

The DVD platform offers a number of advantages over traditional cantilever sensing. It readily supplies large amount of data for statistical analysis facilitating the onset of statistical cantilever based sensing. Moreover, the platform allows for simultaneous measurements of deflection, vibrational amplitude and surface roughness increasing the amount of information to be achieved and consequently the reliability of data. We suggest that cantilever surface roughness is included as an additional parameter in cantilever based sensing as it strongly reflects the uniformity of the surface layer. Also, the nanometre sized laser spot of the PUH facilitates measurements on mechanical sensors with sub-micrometre dimensions. We therefore envision that we in the future will be able to operate ultra-sensitive beams for *i.e.* mass sensing.[35] Finally, the platform integrates a simple and well established method of controlling liquids by centrifugal forces. We hope that our concept will be of use for fundamental as well as more applied studies and we believe that it opens up for a wide range of experiments in, for example, drug screening where reliability and statistical significance of data are crucial.

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References

1 J. K. Gimzewski, C. Gerber, E. Meyer and R. R. Schlittler, Observation of a chemical reaction using a micromechanical sensor, *Chem. Phys. Lett.*, 1994, **217**(5–6), 589.
2 T. Thundat, R. J. Warmack, G. Y. Chen and D. P. Allison, Thermal and ambient-induced deflections of scanning force microscope cantilevers, *Appl. Phys. Lett.*, 1994, **64**(21), 2894.

3 P. S. Waggoner and H. G. Craighead, Micro- and nanomechanical sensors for environmental, chemical, and biological detection, *Lab Chip*, 2007, **7**(10), 1238.

4 R. Datar, et al., Cantilever sensors: nanomechanical tools for diagnostics, *MRS Bull.*, 2009, **34**(6), 449.

5 J. Fritz, Cantilever biosensors, *Analyst*, 2008, **133**(7), 855.

6 S. Singamaneni, et al., Bimaterial microcantilevers as a hybrid sensing platform, *Adv. Mater.*, 2008, **20**(4), 653.

7 C. Ziegler, Cantilever-based biosensors, *Anal. Bioanal. Chem.*, 2004, **379**(7–8), 946.

8 J. W. Ndieyira, et al., Nanomechanical detection of antibiotic mucopeptide binding in a model for superbug drug resistance, *Nat. Nanotechnol.*, 2008, **3**(11), 691.

9 T. Braun, et al., Quantitative time-resolved measurement of membrane protein–ligand interactions using microcantilever array sensors, *Nat. Nanotechnol.*, 2009, **4**(3), 179.

10 J. Mertens, et al., Label-free detection of DNA hybridization based on hydration-induced tension in nucleic acid films, *Nat. Nanotechnol.*, 2008, **3**(5), 301.

11 G. Meyer and N. M. Amer, Novel optical approach to atomic force microscopy, *Appl. Phys. Lett.*, 1988, **53**(12), 1045.

12 G. Binnig, C. F. Quate and C. Gerber, Atomic force microscope, *Phys. Rev. Lett.*, 1986, **56**(9), 930.

13 J. Mertens, M. Alvarez and J. Tamayo, Real-time profile of microcantilevers for sensing applications, *Appl. Phys. Lett.*, 2005, **87** (23).

14 H. P. Lang, et al., Sequential position readout from arrays of micromechanical cantilever sensors, *Appl. Phys. Lett.*, 1998, **72**(3), 383.

15 R. Sandberg, A. Boisen and W. Svendsen, Characterization system for resonant micro- and nanocantilevers, *Rev. Sci. Instrum.*, 2005, **76**(12), 125101.

16 S. H. Lim, D. Raorane, S. Satyanarayana and A. Majumdar, Nano-chemo-mechanical sensor array platform for high-throughput chemical analysis, *Sens. Actuators, B*, 2006, **119**(2), 466.

17 A. Boisen, J. Thaysen, H. Jensenius and O. Hansen, Environmental sensors based on micromachined cantilevers with integrated read-out, *Ultramicroscopy*, 2000, **82**(1–4), 11.

18 G. Yoshikawa, et al., Sub-ppm detection of vapors using piezoresistive microcantilever array sensors, *Nanotechnology*, 2009, **20**(1), 5.

19 J. W. Yi, W. Y. Shih, R. Mutharasan and W. H. Shih, *In situ* cell detection using piezoelectric lead zirconate titanate-stainless steel cantilevers, *J. Appl. Phys.*, 2003, **93**(1), 619.

20 J. H. Lee, et al., Immunoassay of prostate-specific antigen (PSA) using resonant frequency shift of piezoelectric nanomechanical microcantilever, *Biosens. Bioelectron.*, 2005, **20**(10), 2157.

21 G. Shekhawat, S.-H. Tark and V. P. Dravid, MOSFET-embedded microcantilevers for measuring deflection in biomolecular sensors, *Science*, 2006, **311**(5767), 1592.

22 S. H. Tark, et al., Nanomechanoelectronic signal transduction scheme with metal-oxide-semiconductor field-effect transistor-embedded microcantilevers, *Appl. Phys. Lett.*, 2009, **94**(10), 3.

23 Concentris webpage, 2010, www.concentris.ch.

24 F. G. Bosco, et al., Self-aligned cantilever positioning for on-substrate measurements using DVD pickup head, *Microelectron. Eng.*, 2010, **87** (5–8), 708.

25 E. T. Hwu, et al., Simultaneous detection of translational and angular displacements of micromachined elements, *Appl. Phys. Lett.*, 2007, **91** (22), 3.

26 H. J. Butt and M. Jaschke, Calculation of thermal noise in atomic-force microscopy, *Nanotechnology*, 1995, **6**(1), 1.

27 D. C. Duffy, et al., Microfabricated centrifugal microfluidic systems: characterization and multiple enzymatic assays, *Anal. Chem.*, 1999, **71** (20), 4669.

28 M. Madou, et al., Lab on a CD, *Annu. Rev. Biomed. Eng.*, 2006, **8**, 601.

29 B. Ilic, D. Czaplewski, M. Zalalutdinov, H. G. Craighead, P. Neuzil, C. Campagnolo and C. Batt, Single cell detection with micromechanical oscillators, *J. Vac. Sci. Technol., B*, 2001, **19**(6), 2825.

30 W. M. Shu, E. D. Laue and A. A. Seshia, Investigation of biotin–streptavidin binding interactions using microcantilever sensors, *Biosens. Bioelectron.*, 2007, **22**(9–10), 2003.

31 L. Clausen, F. Larsen and H. J. Albrechtsen, Sorption of the herbicide dichlobenil and the metabolite 2,6-dichlorobenzamide on soils and aquifer sediments, *Environ. Sci. Technol.*, 2004, **38**(17), 4510.

32 L. Bruun, et al., A quantitative enzyme-linked immunoassay for the detection of 2,6-dichlorobenzamide (BAM), a degradation product of the herbicide dichlobenil, *J. Immunol. Methods*, 2000, **240**(1–2), 133.

33 J. Tamayo, D. Ramos, J. Mertens and M. Calleja, Effect of the adsorbate stiffness on the resonance response of microcantilever sensors, *Appl. Phys. Lett.*, 2006, **89**(22), 3.

34 A. K. Naik, et al., Towards single-molecule nanomechanical mass spectrometry, *Nat. Nanotechnol.*, 2009, **4**(7), 445.

35 J. Michael, M. J. Lachut and J. E. Sader, Effect of surface stress on the stiffness of cantilever plates, *Phys. Rev. Lett.*, 2007, **99**, 206102.