Micro-electromechanical Systems

DOI: 10.1002/anie.200702894

Making Ultrasensitive Weighing Biocompatible by Placing the Sample within a Resonant Cantilever**

*Philip Tinnefeld**

microchannels · micro-electromechanical systems · resonators · silicon · weighing of biomolecules

Cantilever transducers constitute an emerging class of transducers for physical, chemical, and biological sensors. Generally, a sensor has to provide selective response, which can be obtained, in the case of a chemical sensor, for example, by specific binding to the target analyte. Here, the specificity can be accomplished by a receptor on the sensor surface that captures the analyte from solution. Besides the recognition process, a sensor requires a transduction process, that is, a specific physical process that transduces the molecular recognition event into a measurable, convenient output signal. A sensor can react to different quantities such as temperature, mass, or concentration of chemical or biologically relevant molecules. In recent years, cantilevers have emerged as a set of transducers that can be applied in all areas that are based purely on the transduction of mechanical energy. The key property is that different stimuli can affect the mechanical characteristics of the cantilever transducers which then can be measured comparatively easily.[1] The binding of an analyte to a selective layer on a cantilever transducer can create a change in the surface stress, which leads to the bending of a cantilever. Therefore, cantilevers are often modified only on one side.

The bending of the cantilever transducer can be read out by various modes, for example, optically, through changes in piezoresistance, or by capacitance measurements. As cantilever deflections are often very small, cantilevers can alternatively be operated in a resonant mode to provide higher sensitivity. The working principle of the resonant mode is essentially the same as that of the quartz crystal microbalance (QCM), which was introduced by Sauerbrey 50 years ago.[2] Micro- and nanomechanical resonators can be treated as weakly damped mechanical oscillators that can be described in a simplified form by Hook's law. Both the spring constant k and the total effective mass m^* determine the mechanical resonance frequency ν of the resonator, which is altered upon addition of a mass Δm. Changes in the mass of the resonator translate into shifts of the resonance frequency ν [Eq. (1)].

$$\nu = \frac{1}{2\pi}\sqrt{\frac{k}{m^* + \alpha\Delta m}} \tag{1}$$

α is a numerical constant that depends on the geometric localization of the added mass (typically, $\alpha = 0.24$ for masses on the tip of a rectangular cantilver).[1] This simple equation is often used as a starting point to estimate the mass sensitivity of resonating cantilever sensors, and directly shows that mass sensitivity is governed by the ratio of the mass change to the overall vibrating mass. Accordingly, there is a correlation between the sensitivity of the resonant mode and the weight of the cantilever: the lighter the resonator, the larger the relative mass increase, and the more sensitive is the detection. The correlation also explains the recent miniaturization of the mass sensors through micro- and nanofabrication for the measurement of inertial mass using resonators.[1,3–5] Current micromachined resonators are often cantilevers that are actuated by electrostatic forces, photothermally, or by using piezoresistive displacement transducers.

One of the driving forces for sensor development is still sensitivity, with striking improvements having been made by using nanoelectromechanical, self-sensing cantilevers that enable mass resolution down to about 7 zg (zeptogram, 1 zg = 10^{-21} g).[6] This resolution corresponds to the mass of a single 4 kDa molecule, and weighing processes with single-Dalton resolution might soon be attained.[6] This direction aims to develop the measurement of inertial mass into a form of mass spectrometry that has an enormous dynamic mass range.

Another area of applications is the life sciences. There is a great need for integrated solutions for detecting biomolecules and their interactions in areas as diverse as systems biology, pharmaceutical research, medical diagnostics, environmental monitoring, and several other areas. The key factor for the combination of inertial mass sensing and biomolecular detection is how to make the cantilever device compatible with the conditions required for biomolecular analysis. The problem is that the resolution obtained with mass sensing by mechanical resonators is lost in liquids. There are two reasons for this: the cantilever is strongly damped, which leads to a reduced quality factor (Q). The Q factor describes the rate of energy dissipation relative to the oscillation frequency, or, in other words, the sharpness of the resonance peak. Second, as

[*] Prof. Dr. P. Tinnefeld
Angewandte Physik—Biophysik
Ludwig-Maximilians-Universität
Amalienstrasse 54, 80799 München (Germany)
Fax: (+49) 89-2180-2050
E-mail: philip.tinnefeld@lmu.de

[**] The author is grateful to M. Heilemann, M. Sauer, and H. E. Gaub are thanked for proofreading the manuscript and the Nanosystems Initiative Munich (NIM) is acknowledged for financial support.

the liquid has to be moved by the vibrating cantilever, a larger effective mass results; several approaches have been presented to deal with this viscous damping in solution. The Q factor is, for example, enhanced by incorporating the cantilever in an amplifying feedback loop.[7] However, the sensitivity is still inferior to measurements obtained in a gas atmosphere or in a vacuum. Alternatively, sample preparation can be separated from the measurement, which can be carried out in a vacuum after drying the sample on the cantilever. This "dip and dry" method has, for example, been used to detect individual viruses.[8,9] Certainly, contamination and unspecific residues can be limited in model experiments, but this approach will most likely not become the "gold standard". It also does not allow for real-time measurements.

Recently, Manalis and co-workers introduced a new kind of cantilever, which solves several of the obstacles of resonant mass sensing by a single trick: the liquid containing the analyte is incorporated inside the cantilever and the suspended microfluidic channels used as the resonant cantilevers are surrounded by a vacuum.[10] Figure 1 shows a schematic representation of such a suspended microchannel resonator (SMR). The cantilever beam has dimensions of $200 \times 33 \times 7$ μm (length × width × thickness) and contains a 3×8-μm (height × width) channel.

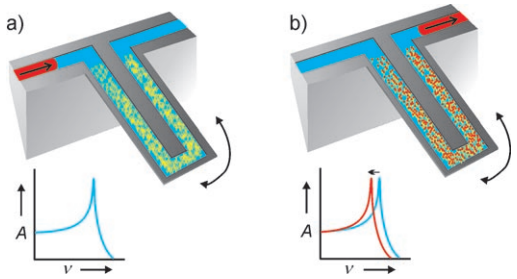

Figure 1. Weighing in suspended microchannel resonators. a) The microchannel resonator translates mass into changes in resonance frequency. b) After binding of analytes (for example, antigens, red) to immobilized antibodies (yellow), the resonance frequency is reduced. A = amplitude, ν = resonant frequency.

The success of the approach is based on the fact that the fluid inside the channel has no measurable effect on the Q factor of the resonator. While the resonance frequency is shifted relative to that of the hollow resonator—as expected from a change in the vibrating mass [see Eq. (1)]—the liquid-filled resonator has an identical and very high Q factor of about 15000. For comparison, Q factors in air are commonly between 10 and 1000 and in solution Q factors are rarely above 10. Accordingly, the SMR has the potential to improve mass resolution by orders of magnitude. It was particularly challenging to enclose the cantilevers directly in a vacuum during the on-chip fabricationfor the development of such a device. This process, however, gives the prospect of cost-effective batch fabrication of wafer-sized systems and offers the possibility for the development of compact portable devices, since the vacuum is supplied with the chip. Buried channels are created by bonding silicon building blocks followed by wafer thinning and dry etching to form suspended microchannels with walls 2–3 μm thick and a 3 μm fluid layer. Subsequent bonding of the silicon layer to pyrex glass was used to create free-standing vacuum-enclosed silicon microchannels. An on-chip getter ensures stability of the low-pressure environment. The cantilever is actuated by electrostatic excitation with electrodes placed inside the vacuum cavity to minimize any effects on the sensitivity. The frequency response of the SMR is detected optically, that is, the amplitude of the vibration is monitored with a laser and a position-sensitive photodetector.

The high sensitivity of this new kind of resonant mass sensor has been demonstrated in a classical assay format.[10] The corresponding frequency shifts caused by the accumulation of molecules inside the chip during the course of the assay preparation are shown in Figure 2a. First, biotin-grafted poly-L-lysine was adsorbed on the cleaned channel walls. Biotinylated antibodies were then linked to the surface through a layer of neutravidin molecules. Subsequently, the interaction with analyte molecules in the solvent was measured through the accumulation of bound molecules in the channel. This was exemplified by detecting different concentrations of goat anti-mouse immunoglobulin-γ (IgG) molecules binding to anti-goat IgG antibodies (Figure 2b). In this configuration, the expected ultimate detection limit for a 30-kDa analyte with a dissociation constant of 1 nM is 1 pM. Mass that is distributed evenly on the inner surfaces can be resolved

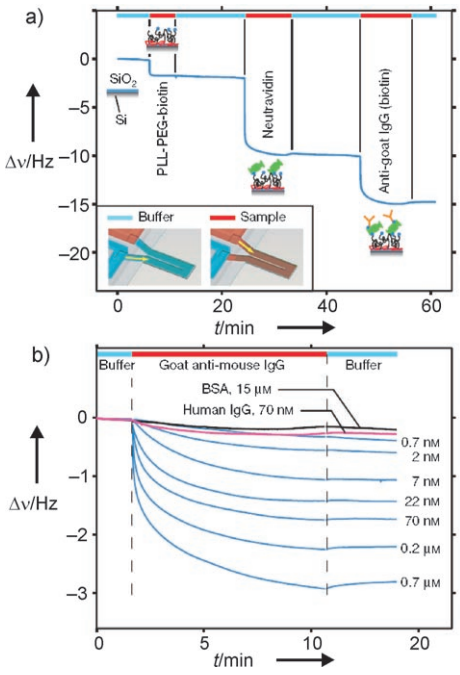

Figure 2. Real-time monitoring of binding in the microchannel. a) On-line monitoring of the cantilever preparation. Each preparation step increases the mass of the channel, thus shifting the resonance frequency. Antibodies are immobilized using electrostatic adsorption of poly-L-lysine (PLL) grafted onto poly(ethyleneglycol)-biotin (PEG-biotin), neutravidin, and biotinylated goat anti-mouse IgG antibodies. b) Change in the resonance frequency after injection of different concentrations of goat anti-mouse IgG. BSA = bovine serum albumin. Figure adapted from Ref. [10] with permission from Macmillan Publishers Ltd, copyright 2007.

to 1 fg of the total mass, with a theoretical limit as low as 1 ag. This lower resolution limit is governed by the thermomechanical background noise of the device. In contrast to alternative weighing methods, such as a quartz crystal microbalance, surface plasmon resonance spectroscopy, or resonant cantilevers optimized for fluid operations or micro-electromechanical (MEMS), acoustic resonators have achieved sensitivity at least three orders of magnitude lower for the detectable total mass. Furthermore, the mass per sensor area, which is useful for comparing the relative concentration sensitivities where there is an unlimited amount of the target compound, is also improved several fold.

It is interesting to consider how the SMR method compares to other mass-based methods and how it relates to other current assay formats.[5,11] A key issue in many assays is label-free detection. Problems with the detection systems are often related to the most sensitive modes of detection in common bioanalyte assays, which nowadays require fluorescent or radioactive labels. Both types of detection necessitate multistep sample preparation and require fairly large volumes. New label-free detection schemes are emerging, with surface plasmon resonance (SPR) being the furthest developed. Commercially available SPR sensors and also quartz crystal microbalances eliminate the need for chemical modification by providing a direct measure of the surface binding on the basis of the intrinsic properties of the molecules. Both methods, however, have their own limitations, and are significantly less sensitive than fluorescence, they require large sample volumes, and they are generally difficult to scale (both down in size and up in number) without degrading the sensitivity. As a result, their utility for biological applications is often limited. Compared to other methods common in diagnostics, such as the enzyme-linked immunosorbant assay (ELISA), similar concentration sensitivities are anticipated with SMRs, but they strongly depend on the specific system, for example, on the mass of the reagents and analytes. Another advantage of SMRs is the high surface to volume ratio, which enables very high efficiency of analyte capture.

There are several interesting and inherent differences between this device and those systems that measure the mass of a sample placed on a cantilever in a vacuum. First, as a result of the sample flowing in the channel, it is actually not the mass of the sample that is directly measured, but the change in the resonance frequency is caused by a change in the mass when the measured particles pass through or bind to the channel and replace the corresponding volume of solvent molecules. For biological molecules, this does not impose a large loss of sensitivity, since proteins, for example, have a mass density of 1.3–1.4 $g\,cm^{-3}$ compared to approximately 1 $g\,cm^{-3}$ for pure buffer. This sensitivity to density changes can also be exploited, for example, to size submicrometer particles. In cases where the density is unknown, this value may be obtained by conducting the measurement in two different carrier solutions to yield both the density of the particles and their volumes. Second, SMR offers direct and intrinsic compatibility with microfluidic systems. Fluids have to be supplied to the SMR and the flow rate has to be controlled precisely. Minute sample volumes are needed. Burg et al. used an autosampler in conjunction with fluid vials to maintain different pressures between the input and output systems.[10]

Besides the impressive results with respect to sensitivity, the SMR method offers a further mode of operation: it can weigh particles in a flow-through mode, whereas all other approaches require surface attachment. Particles that flow through the SMR without binding to the surface will create a signal that depends on the position of the particles along the channel (Figure 3). As a particle approaches the cantilever

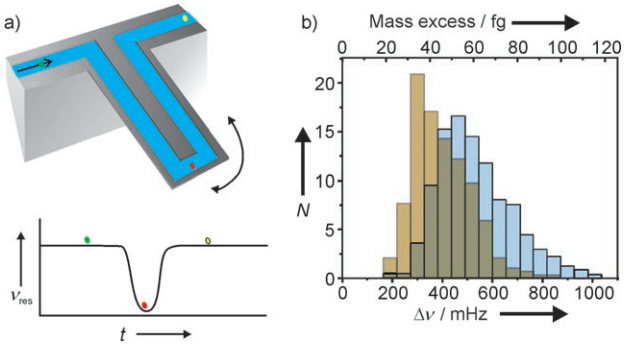

Figure 3. a) Representation of the "flow-through" mode unique to suspended microchannel resonators. The resonance frequency depends on the position of the particle in the channel. The shift in the resonance frequency ν_{res} resulting from the movement of the green, red, and yellow particles are shown below in the form of a ν_{res}–t diagram. The particle mass is determined from the largest frequency change when the particle is at the apex of the cantilever. b) The masses of *E. coli* (brown) and *B. subtilis* (blue) in buffer were measured by passing the bacteria one by one through the resonator. (The Figure was adapted from Ref. [10] with permission from Macmillan Publishers Ltd, copyright 2007).

apex it causes an increasing change in the resonance frequency which subsequently decreases after it has passed the apex. The exact mass excess of the particle can be quantified by the maximum frequency shift induced. This "flow-through" mode has been demonstrated by determining the mass of single gold nanoparticles (100 nm diameter) and polystyrene microparticles (1.1 μm diameter). Low concentrations and strongly reduced flow rates are required so that at most only one particle is in the SMR at a time and to provide the required resolution. The largest error of this mode is induced by the different pathways the particle can assume through the 3-μm wide channel: Particles passing the apex on the outside of the channel induce an 8% larger frequency shift than particles traveling near the inner channel wall. Accordingly, the obtained histograms of size distributions are convoluted by the broadening arising from the different pathways of the particles which hampers precise characterization of the population. As the pathways are not necessarily equally populated, a straightforward deconvolution is not accurate. However, further adaptation of the channels to the specific target and hydrodynamic focusing can strongly improve the accuracy of this measuring mode.

Still, the ability to measure every single particle in a flow-through mode, one after another, represents an entirely new approach to weighing, which resembles optical detection in

chromatography or cytometry. It is this similarity which could lead to revolutionary new applications, for example, mass-based cytometry with the ability to identify and count single cells or small particles. This ability has been demonstrated in the work by Burg et al. in which single bacteria of *Escherichia coli* and *Bacillus subtilis* were weighed, and resulted in broad distributions of (110 ± 30) fg in excess of the displaced buffer for the former and (150 ± 40) fg for the latter (Figure 3b).[10] While this kind of mass sensing clearly achieves the sensitivity to characterize single cells, doubts might be raised as to whether it also provides the specificity required for cell sorting and other cytometry applications. Isn't the mass of a cell dominated by its growth and state of the division process rather than by its species? In fluorescence methods the selectivity is increased by labeling binding molecules with a fluorescent dye, so that detection and selectivity are obtained separately. A labeling approach analogous to fluorescent labeling could also be developed for mass-based signal transduction, for example, cytometry. Instead of the fluorescent label, "heavy" nanoparticles could be developed. Nanoparticles functionalized with specific antibodies could help to distinguish cells of interest from other cells present in a blood sample. Although the approach loses some of its charm through the use of labels, detection with portable and disposable devices could still be cost-effective relative to current optical methods and help to spread the availability of cytometry-related diagnostics. The low throughput of SMR compared to, for example, fluorescence-activated cell sorting (FACS) could be addressed by parallelization of cantilever arrays.

The study by Manalis and co-workers provides thus far the finest method for weighing under biological relevant conditions. It bridges the disciplines of ultrasensitive weighing, microfluidics, and biomolecular analytics. Especially microfluidics, which has the potential to influence many areas of research and technology, could benefit from a new sensing technology that is intrinsically compatible. One reason why microfluidics has not achieved widespread routine use, as proposed several years ago, is related to the difficulty of connecting the microfluidic devices to the outside world—that is sample handling as well as the detection system.[12] The SMR method has the potential to be integrated directly into microfluidic circuits, and detection could be implemented directly on the chip. Future devices will be fine-tuned for their respective applications: larger channels will allow the passage of eukaryotic cells so that specific cells can be counted. This approach should yield cheaper cell counters which are, for example, needed in AIDS monitoring for counting CD4 cells. Small channels, thinner walls, and different channel geometries, on the other hand, will help to increase the detection sensitivity further or to reduce errors in the flow-through of particles, which is related to their pathways close to the apex. The devices could be further developed to avoid optical readout which causes problems with remote applications of portable devices. Self-sensing piezoresistive detection of the oscillation might, therefore, represent an alternative.[3]

Despite the many issues that still have to be addressed in more detail, the work by Manalis and co-workers constitutes a true milestone despite the fact that a "killer application" has not been presented, possibly because many issues have not yet been worked out. It is, for example, difficult to evaluate the selectivity of weighing when the masses of the different objects to be distinguished could not even be determined under the relevant conditions. Questions, such as how the mass density of a single cell changes as it goes through cell division, can now be addressed just by trapping a single cell at the apex of an SMR and observing it over an extended period of time. In this regard, the development of the suspended microchannel resonator actually represents a research achievement, which might soon become a technological success with economic impact.

Published online: September 28, 2007

[1] N. V. Lavrik, M. J. Sepaniak, P. G. Datskos, *Rev. Sci. Instrum.* **2004**, *75*, 2229.
[2] G. Sauerbrey, *Angew. Chem.* **1957**, *69*, 761.
[3] M. Li, H. X. Tang, M. L. Roukes, *Nat. Nanotechnol.* **2007**, *2*, 114.
[4] R. Lucklum, P. Hauptmann, *Anal. Bioanal. Chem.* **2006**, *384*, 667.
[5] T. P. Burg, A. R. Mirza, N. Milovic, C. H. Tsau, G. A. Popescu, J. S. Foster, S. R. Manalis, *J. Microelectromech. Syst.* **2006**, *15*, 1466.
[6] Y. T. Yang, C. Callegari, X. L. Feng, K. L. Ekinci, M. L. Roukes, *Nano Lett.* **2006**, *6*, 583.
[7] A. Mehta, S. Cherian, D. Hedden, T. Thundat, *Appl. Phys. Lett.* **2001**, *78*, 1637.
[8] B. Ilic, Y. Yang, H. G. Craighead, *Appl. Phys. Lett.* **2004**, *85*, 2604.
[9] A. Gupta, D. Akin, R. Bashir, *Appl. Phys. Lett.* **2004**, *84*, 1976.
[10] T. P. Burg, M. Godin, S. M. Knudsen, W. Shen, G. Carlson, J. S. Foster, K. Babcock, S. R. Manalis, *Nature* **2007**, *446*, 1066.
[11] T. P. Burg, S. R. Manalis, *Appl. Phys. Lett.* **2003**, *83*, 2698.
[12] G. M. Whitesides, *Nature* **2006**, *442*, 368.

