

# Liquid-Phase Chemical and Biochemical Detection Using Fully Integrated Magnetically Actuated Complementary Metal Oxide Semiconductor Resonant Cantilever Sensor Systems

Cyril Vančura,<sup>†</sup> Yue Li,<sup>‡</sup> Jan Lichtenberg,<sup>§</sup> Kay-Uwe Kirstein,<sup>||</sup> and Andreas Hierlemann\*<sup>\*</sup>

Physical Electronics Laboratory, Wolfgang-Pauli-Strasse 16, ETH Zurich, 8093 Zürich, Switzerland

Fabien Josse\*

Microsensor Research Laboratory, Department of Electrical and Computer Engineering, Marquette University, Milwaukee, Wisconsin 53201-1881

A novel resonant cantilever sensor system for liquid-phase applications is presented. The monolithic system consists of an array of four electromagnetically actuated cantilevers with transistor-based readout, an analog feedback circuit, and a digital interface. The biochemical sensor chip with a size of 3 mm × 4.5 mm is fabricated in an industrial complementary metal oxide semiconductor (CMOS) process with subsequent CMOS-compatible micromachining. A package, which protects the electrical components and the associated circuitry against liquid exposure, allows for a stable operation of the resonant cantilevers in liquid environments. The device is operated at the fundamental cantilever resonance frequency of ~200 kHz in water with a frequency stability better than 3 Hz. The use of the integrated CMOS resonant cantilever system as a chemical sensor for the detection of volatile organic compounds in liquid environments is demonstrated. Low concentrations of toluene, xylenes, and ethylbenzene in deionized water have been detected by coating the cantilevers with chemically sensitive polymers. The liquid-phase detection of analyte concentrations in the single-ppm range has been achieved. Furthermore, the application of this sensor system to the label-free detection of biomarkers, such as tumor markers, is shown. By functionalizing the cantilevers with anti-prostate-specific antigen antibody (anti-PSA), the corresponding antigen (PSA) has been detected at concentration levels as low as 10 ng/mL in a sample fluid.

In recent years, the interest in biochemical sensors operating in the liquid phase has strongly increased.<sup>1–3</sup> Possible applications of direct chemical and biological sensing devices include the monitoring of chemical contaminants in water and blood analysis.

\* Corresponding authors. E-mail: hierlema@hys.ethz.ch (A.H.); fabien.josse@mu.edu.

<sup>†</sup> Now with Robert Bosch Corp., RTC North America, Palo Alto, CA 94304.

<sup>‡</sup> Now with CSEM, Zurich, Switzerland.

<sup>§</sup> Now with Uwaterc, Hallwil, Switzerland.

<sup>||</sup> Now with Miromico AG, Zurich, Switzerland.

The desired features of such devices are stability, real-time sample detection, and little or no sample pretreatment necessary. Furthermore, most of the possible applications include on-site detection of the analytes. Mass-sensitive or acoustic wave-based devices for liquid-phase chemical and biosensing include, for example, shear-transverse acoustic wave devices,<sup>4</sup> love wave devices,<sup>5</sup> or thickness shear mode resonators,<sup>6</sup> all of which rely on shear-type or horizontal transducer movements to avoid the strong damping of the liquid phase.<sup>7–9</sup> Also, cantilevers have been used for liquid-phase sensing; however, they have been operated in the static mode; i.e., the cantilever deflection upon stress changes in the sensing layer as a consequence of analyte immobilization has been measured.<sup>10,11</sup> In this paper, a monolithic complementary metal oxide semiconductor (CMOS)-based resonant cantilever system for chemical and biological sensing applications in liquid phase is presented.

As has been shown in refs 12–17, resonant cantilevers can be used as gas sensors. Here, the sensor is essentially functioning as a balance, with the resonance frequency depending on the cantilever mass loading. When resonant cantilevers are operated in liquid phase, the physical properties of the liquid environment

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have to be accounted for in addition to the properties of the cantilever and the chemically sensitive coatings. The detection of the cantilever resonance frequency in liquid phase is facilitated by operating the device under closed-loop conditions, i.e., the energy that is dissipated into the viscous liquid has to be provided by the actuation scheme with the appropriate timing to maintain a cantilever oscillation. Moreover, the cantilever has to be very stiff (here 760 N/m) to be able to displace the liquid during its movement. Due to the comparably high density and viscosity of the surrounding medium, the cantilever oscillation is strongly damped, and the cantilever characteristics, such as its resonance frequency and quality factor, also depend on the properties of the fluid. Therefore, it is necessary to include reference cantilevers without receptor or recognition layers into the sensor system, to differentiate between frequency shifts as a consequence of analyte immobilization on the cantilever, and sensor signals that are a consequence of changes in the liquid parameters such as density and viscosity.

To use the cantilevers as liquid-phase chemical sensors, a sensitive layer has been deposited on the resonating cantilevers, which consists, for example, of a polymer that absorbs volatile organic compounds (VOCs) from the surrounding fluid. For these polymeric films, physisorption and bulk dissolution of the analyte molecules within the polymer matrix are the predominant interaction or immobilization mechanisms. The absorption of analyte molecules in the polymeric film on the cantilever leads to a mass increase and concurrently to a decrease of the resonance frequency. The frequency shift is directly proportional to the mass change. In addition to the chemical measurements with polymeric coatings, biologically functionalized cantilevers have been used for biomolecular detection experiments.

## LIQUID-PHASE OPERATION

When a cantilever vibrates in a viscous fluid, the fluid offers a considerable resistance to the motion of the beam, which can be described by an external force acting on the cantilever.<sup>18</sup> This force can be represented by two terms.<sup>19</sup> The first term is a dissipative force per unit length, which results from the fact that the motion of the surrounding fluid is not necessarily in phase with the cantilever motion. This term is called the fluid damping force and is proportional to the cantilever velocity. The second term is proportional to the cantilever acceleration and results from fluid sticking to the surface of the cantilever. Thus, the cantilever drags a specific amount of fluid along, which causes an additional inertial force. By including this external force into the equation of motion of a cantilever beam, the fundamental resonance frequency,  $f_R$ , of a cantilever with length,  $L$ , and mass,  $m$ , vibrating in a viscous

fluid can be written as

$$f_R = \frac{f_0}{\sqrt{1 + (Lg_2)/m}} \sqrt{1 + \frac{1}{2Q_{\text{fluid}}^2}} \quad (1)$$

where  $f_0$  is the natural frequency of the cantilever in vacuum,  $g_2$  (mass per length) is the added fluid mass coefficient, and  $Q_{\text{fluid}}$  is the quality factor of the immersed cantilever. The general expression of the frequency shift associated with an analyte uptake in the polymeric film can be expressed as a function of the cantilever mass variation,  $\Delta m$ , the stiffness variation,  $\Delta k_{\text{eff}}$ , etc. By calculating the derivative of eq 1 and by assuming that the variations are small, the resonance frequency shift,  $\Delta f$ , can be expressed as

$$\frac{\Delta f_R}{f_R} = \frac{\Delta k_{\text{eff}}}{k_{\text{eff}}} + \frac{\Delta Q_{\text{fluid}}}{Q_{\text{fluid}}(Q_{\text{fluid}}^2 - 1)} + \frac{L \cdot \Delta g_2}{2(m + Lg_2)} - \frac{\Delta m}{2m} \left(1 - \frac{Lg_2}{m + Lg_2}\right) \quad (2)$$

Variations of the effective cantilever stiffness can occur as a consequence of changes in the viscoelastic properties of the polymer upon analyte absorption. The interaction of the analyte with the rubbery polymer usually leads to polymer chain rearrangements. The polymer softens and its Young's modulus decreases. Because the Young's moduli of the polymers are 3 orders of magnitude smaller than those of the CMOS layers, the effective stiffness of the cantilever (760 N/m) is dominated by the CMOS layers. Variations of the effective stiffness and analyte-induced variations of the polymer Young's modulus are thus, in most cases, negligible, and so are the analyte-induced variations of the quality factor,  $Q_{\text{fluid}}$ , for low analyte concentrations. The variations of the added fluid mass, caused by changes of the fluid density and viscosity, can be accounted for by using an uncoated reference cantilever since this reference cantilever experiences the same fluid load. Thus, the difference,  $\Delta \tilde{f}_R$ , between the frequency shift of the uncoated reference and the coated sensing cantilever is primarily due to a mass variation,  $\Delta m$ , in the sensitive layer and is given by

$$\Delta \tilde{f}_R = - \frac{\Delta m}{2(m + Lg_2)} f_R \quad (3)$$

The mass variation of the sensitive layer can be expressed as a change in the density of the coating material,  $\Delta \rho_{\text{poly}}$ :

$$\Delta m = Lbh_{\text{poly}} \Delta \rho_{\text{poly}} \quad (4)$$

where  $b$  is the cantilever width and  $h_{\text{poly}}$  is the thickness of the polymer coating layer. Combining eqs 1–3 yields the analyte-induced frequency shift:

$$\Delta f_R = - \frac{f_0}{2m} \frac{1}{(1 + (Lg_2)/m)^{3/2}} \sqrt{1 + \frac{1}{2Q_{\text{fluid}}^2}} (Lbh_{\text{poly}}) \Delta \rho_{\text{poly}} \quad (5)$$

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From eq 5, the gravimetric sensitivity, which is defined as the sensitivity of the cantilever resonance frequency to changes in the polymer density, can be extracted as

$$G = \left| \frac{\Delta f_R}{\Delta \rho_{\text{poly}}} \right| = \frac{f_0}{2m} \frac{1}{(1 + (Lg_2)/m)^{3/2}} \sqrt{1 + \frac{1}{2Q_{\text{fluid}}^2} (Lbh_{\text{poly}})^2} \quad (6)$$

The gravimetric sensitivity is an inherent feature of the cantilever and the medium, in which the cantilever vibrates, and does not take the physisorption process of the analyte into account. It is only dependent on cantilever physical parameters and the damping due to the surrounding liquid.

For small analyte concentrations, a linear relationship between the analyte concentration in the surrounding medium and the analyte concentration in the sensitive layer can be assumed. By introducing the dimensionless polymer–liquid partition coefficient,  $K_{P-L}$ , the change of the polymer density upon a change of the analyte concentration in the liquid environment,  $\Delta c_{\text{liq}}$ , can be written as

$$\Delta \rho_{\text{poly}} = K_{P-L} \Delta c_{\text{liq}} \quad (7)$$

It should be noted that the analyte concentration in the liquid is given in the unit mg/L, which is in water equal to the more common unit ppm. The polymer–liquid partition coefficient describes the analyte distribution between the polymer and the surrounding liquid phase and is related to the polymer–air partition coefficient,  $K_{P-A}$ , characterizing the analyte distribution between air and polymer, via the following equation:

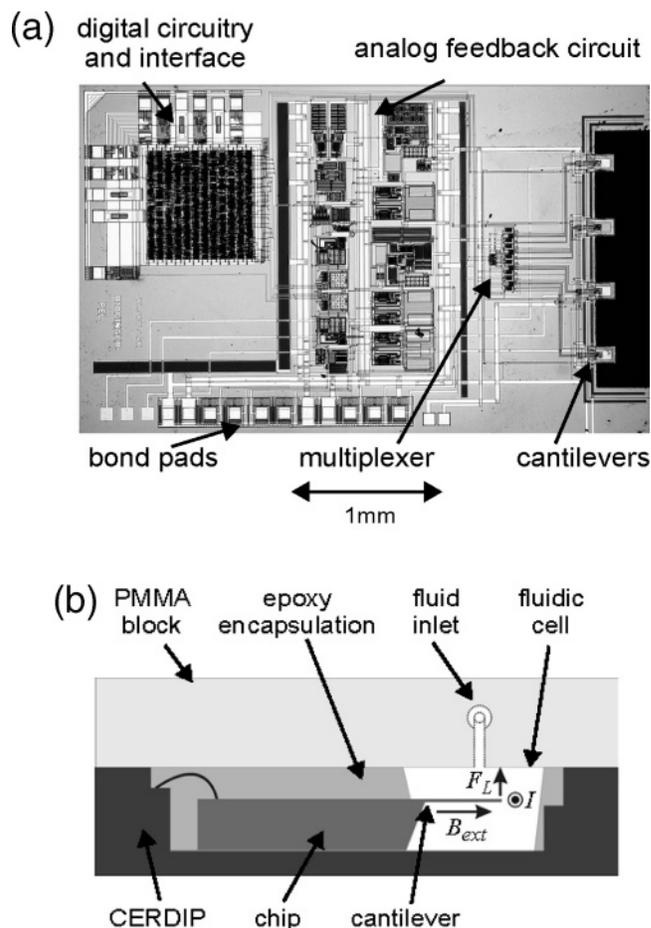
$$K_{P-L} = K_{P-A} / K_{L-A} \quad (8)$$

where  $K_{L-A}$  is the liquid–air partition coefficient. The analyte partitioning into the polymer matrix is dependent on different parameters, such as the volatility of the vapor, the operating temperature, and the interaction forces between the polymer and the analyte.<sup>20</sup> Using eqs 6–8, the chemical sensitivity,  $S_{\text{liq}}$ , of a polymer-coated cantilever operating in liquid environments can be written as

$$S_{\text{liq}} = \left| \frac{\Delta f_R}{\Delta c_{\text{liq}}} \right| = \frac{f_R}{2m} \frac{Lbh_{\text{poly}}}{(1 + (Lg_2)/m)} K_{P-L} \quad (9)$$

## SYSTEM DESIGN

**Monolithic Sensor System** The monolithic sensor system used in this work for the liquid-phase measurements is shown in Figure 1a. It is fabricated using a CMOS process combined with post-CMOS micromachining. The single-chip system includes four cantilevers, a multiplexer, an analog feedback circuit, and digital signal conditioning and processing circuitry. The four integrated cantilevers feature electromagnetic actuation and transistor-based



**Figure 1.** (a) Micrograph of the sensor chip for liquid-phase measurements featuring four cantilevers monolithically integrated with analog and digital circuitry. (b) Cross sectional schematic of the packaged cantilever chip.<sup>21</sup> The direction of the current,  $I$ , at the cantilever tip is indicated.

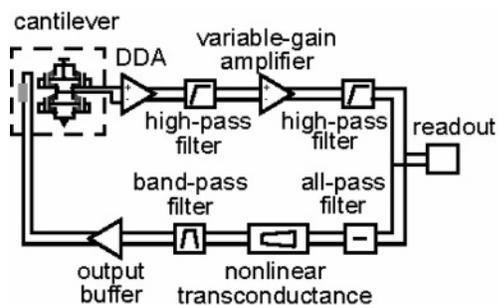
readout.<sup>22</sup> For the electromagnetic actuation, a current path with eight loops, realized by using the two metal layers of the CMOS process, is integrated along the edges of the cantilever (see Figure 1b). A static, external magnetic field is oriented in parallel to the cantilever axis so that a Lorentz force perpendicular to the cantilever plane is generated by a current through the metal path. The Lorentz force then causes a transverse deflection of the cantilever, since it is acting on the cantilever tip, where the metal lines, and, therefore, the currents, are oriented perpendicularly to the magnetic field (see Figure 1b). By applying a sinusoidal current, a transverse oscillation of the cantilever can be excited. The cantilever vibration is detected by a stress-sensitive Wheatstone bridge integrated on the cantilever, which consists of four diode-connected PMOS transistors. The transistor source-drain currents are dependent on the mechanical stress in the transistor channel region.<sup>23</sup> The source-drain currents of these stress-sensitive transistors are controlled by the resistive region of the channel, when operated in strong inversion in either the linear or in the saturation regime. The Wheatstone bridge is located at

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**Figure 2.** Block diagram of the integrated feedback circuit for liquid-phase applications.

the clamped edge of the cantilever, where the bending and, consequently, the stress are maximal, so that a large output signal can be achieved. In comparison to a Wheatstone bridge consisting of diffused resistors<sup>24</sup> (resistive load below 950  $\Omega$ ), the transistor configuration as described above needs less space and shows a higher resistive load (20 k $\Omega$ ) to the power supply. This leads to less power dissipation on the cantilever and, hence, to a lower temperature increase of the sensitive layer on the cantilever.

Only one cantilever is connected to the analog feedback circuitry and operated at a time. Each vibrating cantilever can be addressed by means of a multiplexer and then serves as the frequency-determining element of the feedback loop. The cantilever selection as well as the programming of the integrated phase shifter (band-pass filter) is done with a PC connected to the chip via the on-chip interface.

**Feedback Operation.** To facilitate the operation of the sensor system, a dedicated oscillator circuit with the cantilever as the frequency-determining element has been integrated on the same substrate as a feedback circuit. Thus, the cantilever is operated in self-oscillation, and its resonance frequency can be directly measured.

A block diagram of the sensor system for liquid-phase applications is shown in Figure 2. The output signal of the piezoresistive Wheatstone bridge is amplified in two stages and high-pass filtered to remove the offset coming from the mismatch of the bridge transistors. The first amplifier is a low-noise differential difference amplifier (DDA) based on two operational amplifiers. The second amplification stage is a DDA with variable gain, which is adjustable via a bias current. The following all-pass filter enables the adjustment of the phase and, thus, is needed to meet the phase oscillation condition. The key element of the feedback circuit is a nonlinear transconductance, which is based on a nonlinear, amplitude-limiting conductance.<sup>25</sup> This stage of the feedback circuit is necessary to meet the gain oscillation condition. During the operation in self-oscillation, the nonlinear transconductance adjusts the loop gain on-chip to 1. The gain of this stage decreases with increasing input signal magnitude to avoid the saturation of the overall feedback system during operation. Afterward, the signal is fed via a band-pass filter and a class-AB buffer to the current path on the cantilever. The buffer is needed to drive the low resistive load of the current path. The overall feedback loop features a fully differential architecture for better suppression of

noise and interferences, e.g., from the supply lines and for a reduction of the signal distortion as a consequence of nonlinear effects in the circuitry. During operation, the system oscillates at the mechanical resonance frequency of the cantilever.

**Frequency Monitoring.** During operation, the frequency can be monitored either with a 24-bit counter integrated on chip or with an external counter. The integrated counter in the digital core of the chip can be operated in two different modes: (a) in the triggered mode or (b) in the gated mode. In the triggered mode, the I<sup>2</sup>C “read” command is used to gate the counter. With each read command the counter gives the present counter value. The frequency can be calculated by dividing the difference of two consecutive counter values by their time difference. In the gated mode, the time between two consecutive output values is determined by an external gate signal. Again, the frequency can be calculated by dividing the difference of two consecutive counter values by their time difference, which is, in this case, the gate time. Alternatively, the resonance frequency can be measured with an external counter. For this purpose, the analog output of the feedback circuit is probed with a differential probe (Tektronix, P6246). Most of the presented measurements have been performed with the external counter.

## FABRICATION

**Post-CMOS Micromachining.** The device presented in this paper has been fabricated in a modified industrial CMOS process in combination with post-CMOS micromachining.<sup>26</sup> The process used here is a 0.8- $\mu\text{m}$  double-metal, double-poly-CMOS process as provided by the manufacturer Austriamicrosystems, Untertremstatten, Austria. After the completion of the CMOS process, the cantilevers were released by two postprocessing steps that are conducted on wafer level. First, silicon membranes were formed by an anisotropic wet etching of the bulk silicon in KOH from the wafer backside. To ensure a defined membrane thickness, an electrochemical etch-stop technique<sup>27,28</sup> was used so that the etching stops at the n-well, which was created by ion implantation during the CMOS process flow. Thereafter, the cantilevers were released by reactive-ion etching (RIE) from the wafer front side. The membrane around the designated cantilever shape was removed by front-side RIE of silicon oxide with CHF<sub>3</sub> and CF<sub>4</sub>, and the silicon was removed with SF<sub>6</sub>. The resulting cantilevers are composed of the dielectric layers of the CMOS process on top of a silicon layer (n-well) that has a thickness of  $\sim 5 \mu\text{m}$ . The thicknesses of the silicon oxide and the silicon nitride layer are 2.2 and 1  $\mu\text{m}$ , respectively. The total cantilever thickness is 8.2  $\mu\text{m}$ .

**Chip Dicing.** In order to use the sensor devices in liquid environments, it was necessary to fabricate free-standing cantilevers at one end of the chip that could be fully immersed in the fluid so that special measures had to be taken not to damage the cantilevers during dicing. As the dicing line is in proximity (distance 350  $\mu\text{m}$ ) to the free-standing cantilevers, the water jet for the blade cooling of the dicing saw had to be turned down to

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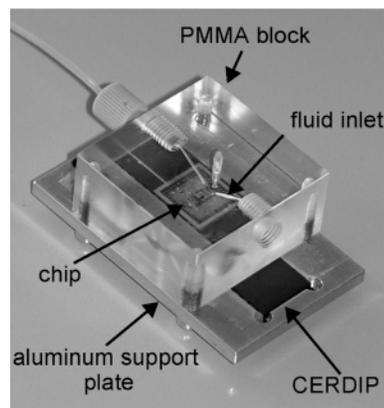
a minimum. Additionally, the cantilevers had to be supported from the back side and protected against dicing debris from the front side. The latter is necessary, since particle contamination cannot be mechanically removed due to the fragility of the cantilevers. The back-side support of the cantilevers was realized as a thick layer of photoresist (Micro Posit S1828, Rohm and Haas Electronic Materials, Coventry, UK), which was either painted or spun onto the wafer prior to the cantilever release in the last RIE step. After the RIE, the front side of the wafer can be protected with an additional layer of photoresist. Another method to protect the wafer front side was to use an adhesive foil. In order to avoid any fracture of the cantilevers when the foil was removed, an Adwill P-5780 foil, manufactured by Lintec Corp.<sup>29</sup> was used. This foil can be rendered nonadhesive by UV light exposure and can be removed after subsequent heating.

### SYSTEM PACKAGING

An electromagnet or a permanent magnet is needed to generate the permanent magnetic field for cantilever actuation. For the integration of the sensor in a portable system, the latter option is better with regard to costs and power consumption. A variety of different magnets have been tested for their capability to provide the magnetic field strength necessary for a stable oscillation of the cantilever in liquid-phase applications. The system packaging requirements are much more stringent for liquid-phase applications than for operation in air:<sup>6</sup> (a) all electrical connections, as well as the integrated circuitry have to be isolated from the conducting fluids; (b) a fluidic cell has to be fabricated around the cantilevers to allow for liquid handling; (c) a permanent magnet has to be included into the package for the electromagnetic actuation of the cantilever. The cantilever sensing system was successfully operated in liquid with four small neodymium disk magnets (diameter 1.5 mm, height 0.8 mm, Northwest Magnet, Inc., Portland, OR) mounted above the cantilevers in the covering PMMA block. Most of the characterizations and measurements in the liquid phase presented in this paper have been performed using a large external tunable electromagnet, which reduced the packaging complexity and facilitated experiments at different magnetic field strength. The diced chips were mounted on standard ceramic dual-in-line packages (CERDIPs) and electrically connected via wire bonding. The bond wires and the integrated electronics were protected using a UV-curable epoxy (OG116, Epoxy Technology, Inc., Billerica, MA), in order to avoid short-circuiting of the electrical connections in the conducting fluids. The epoxy glue can be patterned due to its high viscosity (30 000 cP), which is necessary to avoid any accidental coverage of the cantilevers. During the epoxy processing, the fluidic cell around the cantilevers was formed using a mold, which was placed into the CERDIP before the epoxy was dispensed. The inlet and outlet of the fluidic cell were machined into a PMMA block, which was mounted on top of the CERDIP (see Figure 3).

### EXPERIMENTAL SECTION

**Deposition of Chemically Sensitive Polymer Films.** After the completion of the post-CMOS micromachining steps and after packaging, polymeric films were deposited on the cantilever structures by spray-coating using an airbrush (Badger, model 200-



**Figure 3.** Photograph of the packaged single-chip sensor system with mounted PMMA block for fluid handling.

F, Franklin Park, IL) and shadow masks. The usage of shadow masks allows for coating of the four cantilevers of the array with different polymers or differently thick layers of the same polymer. A wide range of partial selectivities and sorption properties can be covered by careful selection of the polymeric coating materials. To prove the feasibility of the cantilever sensor system for the detection of VOCs in water, two polymers, poly(epichlorohydrin) (PECH; Sigma Aldrich, Switzerland), and poly(isobutene) (PIB; Sigma Aldrich, Switzerland) were chosen. For spraying, the polymers were dissolved in dichloromethane or chloroform (concentrations between 2.5 and 10 mg/mL), and nitrogen was used as a carrier gas. The airbrush was fixed at a distance of 10 cm from the chip surface.

**Solution Preparation.** Aqueous analyte solutions (600 ppm) of ethylbenzene, the xylenes, and toluene were prepared by dosing the appropriate amount of analyte into a large volumetric flask, which had been filled to capacity with a premeasured volume of deionized water to ensure a minimization of the headspace in the flask. The flask was immediately closed after filling. The resulting solutions were then vigorously shaken overnight to ensure an optimal mixing. Extreme care was taken to minimize any volatilization of the analytes from the solution. The respective solution was filled into the glass syringe of the fluidic setup immediately before the measurement. It is apparent that these concentrations lead to supersaturated analyte solutions as the solubilities in water of the respective analytes are 530 ppm for toluene, 130 ppm for xylenes, and 170 ppm for ethylbenzene. But by minimizing the time span between mixing the liquids and the start of the experiment, a phase separation of analyte and water could be avoided.

**Experimental Setup and Procedures.** After packaging, the sensor systems in the CERDIPs with the attached fluidic cell were mounted onto a printed circuit board (PCB), which provided all the necessary biasing and interfacing for the operation of the sensor chip. As already mentioned, most of the measurements in fluids have been performed with a tunable electromagnet in order to have a maximum of experimental freedom. The PCB was placed between the two poles of the electromagnet. The magnetic field strength was measured with a Gauss-meter (Gauss-meter 450, Lake Shore Cryotronics Inc., Westerville, OH) and a Hall probe (MMT-6J02-VG, Lake Shore Cryotronics Inc., Westerville, OH), which was placed next to the fluidic cell. If not otherwise stated,

(29) <http://www.lintec.co.jp>.

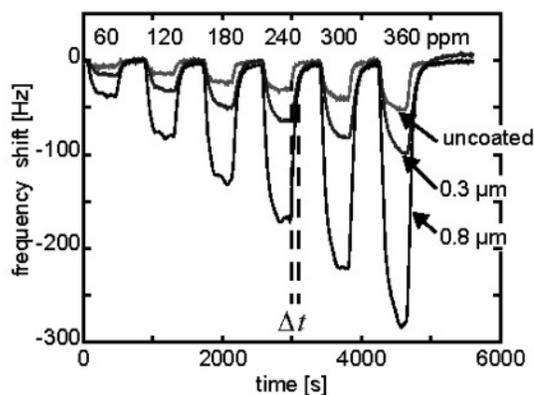
the measurements were performed at a magnetic field strength of 300 mT.

The fluidic cell was connected to two computer-controlled syringe pumps (PicoPlus and 11Plus, Harvard Apparatus, Holliston, MA) via commercial Teflon tubings (outer diameter of  $1/16$  in., Upchurch Scientific, Oak Harbor, WA) and a Y-connector. The glass syringes were purchased from ILS (Innovative Labor Systeme GmbH, Stützerbach, Germany) and feature a volume of 10 mL. For the detection of VOCs in water, various analyte concentrations in aqueous solution were prepared using the two syringe pumps, one loaded with pure, deionized water and the other loaded with a 600 ppm analyte solution. The measurements were performed at a constant flow of  $100 \mu\text{L}/\text{min}$  through the measurement chamber. The analyte concentrations were adjusted by setting different flow velocities for the two pumps. A monitoring of the cantilever resonance frequency upon operating the liquid flow indicated a negligible cantilever perturbation at the selected flow rate. A typical measurement consisted of alternating exposures to pure, deionized water and analyte–water mixtures. In most cases, the analyte concentrations were ramped up starting from the lowest concentration. The resonance frequency of the cantilevers was continuously measured using an external frequency counter (HP 53131 A) with a gate time between 1 and 3 s.

## RESULTS AND DISCUSSION

**Sensor Response.** A typical measurement run with polymer-coated cantilevers was started by pumping pure DI water for at least 1 min through the measurement chamber, which hosts the sensor chip. Then, the chip was exposed to a 240 mg/L analyte solution for 7 min. Next, the measurement cell was again purged with pure DI water for 5 min to achieve a stable baseline. This process, known as conditioning of the polymeric film on the cantilever, improves device stability and reproducibility of the measurement results. Then, during the measurement series, the sensor chip was alternately exposed to analyte solutions of specific concentrations and DI water. The short-term frequency stability of the system during such measurements cycles was determined to be 3 Hz. It should be noted that values as low as 0.03 Hz have been reported<sup>17</sup> for a similar system operating in air. This reduction of the frequency stability is a consequence of the lower cantilever quality factor in water ( $Q_{\text{water}} \approx 10$ ) as compared to the operation in air ( $Q_{\text{air}} \approx 1000$ ).

Figure 4 shows the response of two PECH-coated and one uncoated cantilever after conditioning upon exposure to different concentrations of ethylbenzene, ranging from 60 to 300 mg/L. As can be seen, all three cantilevers show a distinct response upon the presence of the analyte solution. In the case of the uncoated cantilever, this response is due to changes of the fluid density and viscosity caused by the addition of ethylbenzene to the DI water. The frequency response measured with the coated cantilevers consequently includes both, mass loading of the polymer as well as unspecific signals as a consequence of density and viscosity changes. As expected, the partitioning of the analyte into the polymer matrix is fully reversible, and the cantilever resonance frequency returns to its initial value after purging the fluidic cell with pure water. From the volume of the measurement chamber ( $16 \mu\text{L}$ ) and the flow rate of  $100 \mu\text{L}/\text{min}$ , it can be deduced that a complete exchange of the liquid volume of the cell takes  $\sim 30$  s.



**Figure 4.** Response of three different cantilevers exposed to various concentrations of ethylbenzene in aqueous solution. Two cantilevers are coated with PECH (0.3 and  $0.8 \mu\text{m}$  polymer layer thickness); one cantilever is uncoated.

When the cell is being purged with DI water, it can be observed that the uncoated cantilever returns much faster to the baseline than the coated cantilevers. The time difference,  $\Delta t$ , as indicated in Figure 4 is  $\sim 180$  s, so that it can be concluded that the time constant of the sensor response is dominated by the polymer/analyte interaction and not by the liquid exchange in the fluidic cell. The observed frequency shift is linearly correlated to the analyte concentration within the measured concentration range. The small observed deviations from linearity are most probably a consequence of the analyte volatility and of fluctuations in the sample preparation procedure.

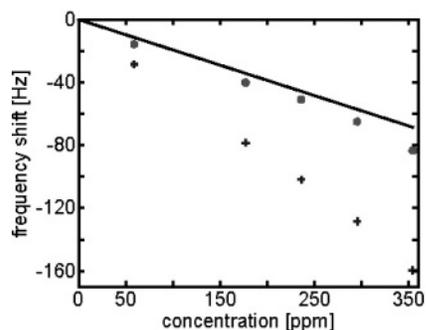
**Mass Loading and Fluid Property Contributions to the Sensor Signal.** In the gas phase, the mass loading due to an uptake of the analyte in the polymer is the main contribution to the sensor response.<sup>30</sup> Since the stiffness of the cantilever is dominated by the CMOS materials, slight changes of the elastic properties of the polymer due to analyte absorption can be neglected. In the liquid phase, the sensor signal is not only affected by the analyte uptake in the sensitive layer but also by changes in the fluid properties. From Figure 4, it can be seen that the uncoated reference cantilever also exhibits a significant frequency shift attributed to analyte-induced changes in the fluid density and viscosity. By calculating the differential sensor signal (difference between the frequency shift of a coated and an uncoated cantilever), the contribution of the polymer/analyte interaction can be extracted.

In ref 31, the effect of volatile organic compound absorption in the polymer is described by a swelling of the polymer and an increase of the polymer density. This approach has been extended to analyte absorption into polymers from liquid phase in ref 32. The effects of analyte and water absorption in the polymer are assumed to be additive for the range of tested analyte concentrations. The approach of ref 32 was also used in this paper to calculate the analyte-induced polymer swelling and density increase. Utilizing these two parameters, the change in the polymer mass upon analyte absorption was then calculated.

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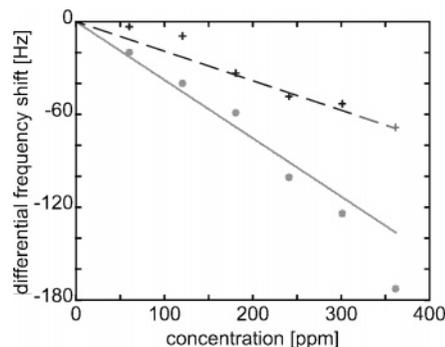
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**Figure 5.** Measured frequency shift (+) and differential sensor response (\*) of a cantilever coated with  $0.3 \mu\text{m}$  of PIB in aqueous liquid phase upon exposure to five different concentrations of ethylbenzene. The solid line represents the calculated frequency shifts based on the mass loading as derived from the aqueous solution-polymer partitioning.

Figure 5 shows the measured frequency shift and the differential sensor response of a cantilever coated with  $0.3 \mu\text{m}$  of PIB upon the absorption of five different concentrations of ethylbenzene in water. As can be seen, the polymer-induced frequency shift at this thickness amounts only to  $\sim 50\%$  of the total signal. The other  $50\%$  of the signal are caused by changes in the fluid properties.

Figure 5 also depicts the theoretical frequency shifts calculated using eq 9 and accounting for the polymer density and thickness increase according to ref 32. A water uptake of 200 ppm in the polymer was assumed in concordance with reported values<sup>33</sup> and was taken into account in the calculation of the polymer thickness and density. The analyte concentration in the polymer was calculated with a liquid-phase partition coefficient of  $K_{\text{P-L}}^{\text{ethylbenzene}} = 464$  for PIB and ethylbenzene.<sup>34</sup> As can be seen, the differential sensor signal is in good agreement with the calculated frequency shifts. The small difference between the measurements and the predicted values could be a result of conformational changes in the polymer matrix upon analyte absorption. This so-called dilution or plasticization<sup>20</sup> of the polymer entails a polymer softening and, consequently, a decrease of its Young's modulus so that the stiffness of the composite cantilever decreases a little bit, which, in turn, may lead to a slight decrease of the cantilever resonance frequency. Such analyte-induced changes of the viscoelastic properties of the polymer have been demonstrated to provide significant contributions to the frequency changes of polymer-coated, shear horizontal surface acoustic wave devices operated in water. The viscoelastic contribution has been reported to be between three and four times larger than the mass-loading contribution.<sup>32</sup> None of the microcantilever measurements presented here indicates that the polymer softening has such a substantial effect also on the cantilever response. This is most probably due to the fact that the cantilever stiffness is determined by the material properties of the CMOS layers and is only slightly influenced by the polymer as a systematic difference between the differential sensor response and the predicted frequency shift (see Figure 5) was not observed in all the measurements. Another reason might be the much lower operating frequency of the



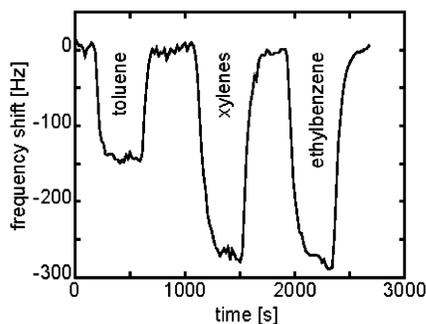
**Figure 6.** Differential sensor response of two cantilevers coated with PIB layers of different thickness (\*,  $0.6 \mu\text{m}$ ; +,  $0.3 \mu\text{m}$ ) upon absorption of different concentrations of xylenes. The solid and dashed lines represent the calculated frequency shift for the two layers.

cantilever in comparison to surface acoustic wave devices and the difference of the relative displacements of the polymer layers in the two different devices. In the case of the cantilever, the polymer film essentially moves as a single entity with the cantilever that undergoes only a minor bending of a few nanometers in amplitude. For acoustic wave devices, the movement occurs in the plane of the device with a wavelength much smaller than the lateral extent of the film, which results in a differential displacement in the polymer film due to the propagating waves. It is assumed that such a differential movement within the polymer is much more sensitive to changes of the viscoelastic properties of the film than the slight bending occurring during the movement of the coated resonant cantilever. A definitive statement regarding viscoelastic contributions is not yet possible. Obviously, the frequency variations of the sensor system (cantilever and integrated circuitry) and the variability of the measurement setup are too large to allow for a detailed analysis of small signal contributions. A more stable system would be necessary to investigate the effects of viscoelastic contributions. Additionally, a simultaneous monitoring of the resonance frequency and the quality factor would be necessary, as the quality factor is expected to decrease as a consequence of the softening of the polymer with increasing analyte concentration.

**Effects of the Polymer Layer Thickness.** Figure 6 shows the differential sensor responses of two cantilevers, which are coated with  $0.3$  and  $0.6 \mu\text{m}$  of PIB, upon the absorption of different concentrations of xylenes. As expected, the cantilever with the thicker polymer coating is roughly twice as sensitive as the cantilever with the thinner layer. The sensitivities are  $S_{\text{liq}} = 0.19 \text{ Hz/ppm}$  and  $S_{\text{liq}} = 0.38 \text{ Hz/ppm}$ , respectively. Due to the increased polymer volume, the amount of absorbed analyte and, consequently, the mass loading are larger. In ref 3, it was shown that the sensitivity in the gas phase is proportional to the polymer layer thickness at such small polymer thicknesses since the lowering of the base resonance frequency upon polymer deposition can be neglected. In the liquid phase, this effect is even more pronounced, as the resonance frequency is, in contrast to the gas-phase application, dominated by the liquid loading and not by the polymer loading since the effective mass of the cantilever is larger than that in the gas phase due to the fluid being dragged along with the cantilever. The shift of the base resonance frequency due to the polymer coating is, therefore, even smaller. Another effect that has to be considered in liquid environments is that the

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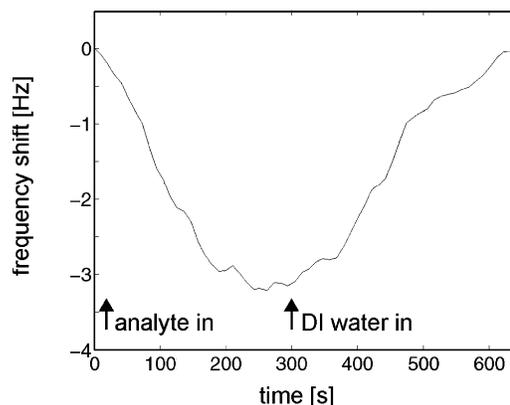
**Figure 7.** Frequency response of a PIB-coated cantilever upon exposure to three different nonpolar analytes (toluene, xylene, ethylbenzene) at a concentration of 300 ppm in water.

sensitivity also decreases as the quality factor decreases due to the liquid loading.

**Sensor Sensitivity and Limit of Detection.** A careful selection of the sensitive coatings is necessary to enable the detection and discrimination of different analytes. Polymer-coated sensors are only partially selective, owing to the physisorption-type interaction mechanism between analyte and polymer. The selectivity of a sensor system can be improved by using a sensor array featuring sensors with different sensitive layers,<sup>24,35</sup> which allows for the discrimination of multiple analytes by applying mathematical methods, such as principal component analysis to the response patterns. An effective criterion for the selection of the polymer coating is the respective polymer/analyte partition coefficient.

Figure 7 shows the frequency response of a PIB-coated cantilever upon the absorption of 300 ppm toluene, xylenes, and ethylbenzene in water. The measured frequency shifts are in agreement with a trend predicted from the partition coefficients ( $K_{P-L}^{\text{toluene}} = 141$ ,  $K_{P-L}^{\text{xylenes}} = 449$ , and  $K_{P-L}^{\text{ethylbenzene}} = 464$ ).<sup>34</sup> Ethylbenzene causes a slightly larger frequency shift than the xylenes, whereas toluene produces a clearly smaller signal. Nevertheless, it should be noted that the signals measured for toluene were systematically larger than the prediction based on the partition coefficients. The reason for this discrepancy is not clear.

Figure 8 shows the response of a cantilever with a 0.8- $\mu\text{m}$ -thick PECH layer upon exposure to an aqueous 6 ppm xylene solution, which corresponds to  $\sim 5\%$  of the analyte saturation solubility in water. The noise has been reduced by filtering the data after the measurement with a polynomial smoothing filter (Savitzky–Golay filter, window size 11) in MATLAB. Calculations reveal that the observed frequency shift corresponds to an added mass of 50 pg, which corresponds to a mass per area of  $2.4 \times 10^{-3} \text{ kg/m}^2$ . Here it was assumed that the frequency shift is only due to mass loading since the uncoated reference cantilever did not exhibit a detectable frequency shift. It should be noted that this value is not the limit of detection of the sensor system, which is typically determined as three times the noise floor, but it has been determined from filtered data. Without filtering the data, the limit of detection is  $7.1 \times 10^{-3} \text{ kg/m}^2$  in mass per area units. The measurement shows the lowest measurable concentration with the current chip design at this polymer thickness. The limit of



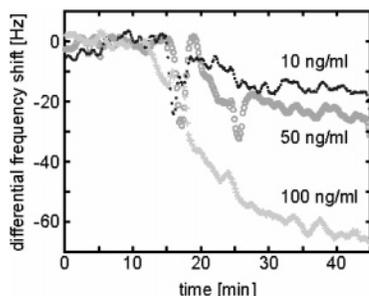
**Figure 8.** Response of a cantilever coated with a 0.8- $\mu\text{m}$ -thick layer of PECH upon exposure to 6 ppm xylene in water.

detection can be further reduced by using thicker polymer layers, decreasing the noise of the sensor system, and optimizing the measurement setup. In ref 32, measurements of 1 ppm ethylbenzene, xylenes, and toluene with a PECH-coated shear-horizontal acoustic wave sensor were presented. Based on these measurements and the noise floor of the device, the limit of detection was estimated to be in the 10 ppb range for the three analytes. The lower limit of detection of this device as compared to the cantilever sensing system can be partly explained by its sensitivity to viscoelastic changes in the polymer layer. The viscoelastic changes in the polymer significantly increase the frequency shift over the pure mass loading signal. Furthermore, the device seems to exhibit a lower noise floor due to its horizontal movement in the water. A drawback of the shear horizontal acoustic wave system is that cannot be integrated and external circuitry is needed to read out the sensor signal.

**Biomarker Detection.** In a next step, the presented monolithic cantilever sensing system has been used to demonstrate its capability for label-free biological detection. As model assay, the binding of prostate-specific antigen (PSA) to anti-PSA was chosen. PSA is a tumor marker for prostate cancer, which is one of the most common forms of male cancer. The successful detection of PSA has been demonstrated in ref 36 by applying various PSA concentrations in a buffer solution to functionalized cantilever arrays that were operated in the static mode. The tests with the single-chip cantilever sensor system have shown that the detection of PSA in a sample fluid is also possible using resonant cantilevers. Figure 9 shows the differential frequency shift of the sensor system for three different concentrations of PSA at clinically significant levels. The packaged chips were first cleaned with 2 M NaOH and 2M HCl (1 min each). Thereafter, the chip was rinsed with deionized water and ethanol. Next, the sensing cantilever was functionalized with protein A linker molecules (protein A, Sigma Aldrich, Switzerland). This was done by immersing one cantilever into a 1 mg/mL protein-A solution and incubating the chip for 12 h at 4 °C. Thereafter, the packaged chip was again rinsed with deionized water. After the rinsing, the sensing cantilever was functionalized with PSA antibodies by immersing it into an anti-PSA solution (PSA 10, purified, CanAg Diagnostics AB, Gothenburg, Sweden) and incubating the chip

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**Figure 9.** Differential frequency response of the cantilever sensor system upon exposure to three different concentrations of PSA.

for 2 h at room temperature. Finally, the chip was rinsed with phosphate-buffered saline (PBS, pH 7.4). Special care was taken not to functionalize the three other cantilevers, which served as uncoated reference devices.

After the functionalization procedure, the packaged cantilever chip was mounted into the fluidic setup described earlier. One syringe was loaded with PBS solution, the other with a PSA solution (PSA Control solution, 400 ng/mL, CanAg Diagnostics AB) diluted with PBS to the respective concentrations. The experiment was conducted under stop-flow conditions with a flow rate of 10  $\mu$ L/min. First, the fluidic cell was filled with PBS buffer and the cantilever frequency was monitored until a stable baseline was reached. Then, the external syringe pump was turned on for 10 min at the selected flow rate in order to inject the PSA sample fluid. As can be seen in Figure 9, the sensor shows a distinct response to all three PSA concentrations and the results show that a PSA concentration of 10 ng/mL in PBS can actually be detected. The measurements were all performed with the same device, which was regenerated with NaOH and HCl after each measurement. This is, to the best of our knowledge, the first successful detection of a biomarker in liquid environments using a monolithic resonant cantilever chip with integrated actuation and readout. The frequency data were, again, filtered using MATLAB (Savitzky–Golay filter, window size 11). The signal distortion during the sample injection is most probably caused by the pumping. For clinical applications, the detection of PSA levels as low as 2 ng/mL in serum samples from real blood is necessary. The presented results are very promising and indicate that this may be achieved with a fully integrated cantilever sensing system. It is estimated that for a clinical application the signal-to-noise ratio would have to be improved by at least a factor of 3. Possible improvements may result from an optimization of the functionalization protocol to achieve a higher and more uniform surface density of the anti-PSA molecules and from the usage of an even smaller fluidic cell to shorten the system response and analysis time.

## CONCLUSIONS

A CMOS resonant cantilever system for biochemical applications has been presented. The integrated sensor system is fabricated in an industrial CMOS process, and the cantilevers are released using subsequent CMOS-compatible micromachining. Electromagnetic actuation is used to drive the cantilevers, and the vibration is read out by means of integrated, piezoresistive PMOS transistors. The cantilevers consist of a stack of single-crystal silicon, silicon oxide, and silicon nitride layers. Utilizing CMOS technology, which is the most common technology for the fabrication of integrated circuits, allows for the monolithic integration of the sensor elements with dedicated analog and digital circuitry.

The monolithic sensing system has been used for the detection of volatile organic compounds in liquid environments. Here, the packaging strategy is very important. The integrated circuitry and the electrical connections have to be isolated from the electrically conducting fluid. At the same time, the packaging has to incorporate a fluidic cell around the cantilevers, which allows for a confinement of the sample fluid to the region of the sensing elements. The operating frequency of the cantilever is  $\sim$ 200 kHz in water. A frequency stability better than 3 Hz was achieved, owing to the integrated analog feedback circuit. Two different polymers, poly(isobutene) and poly(epichlorohydrine) have been used for VOC detection in water. The response of the polymer-coated cantilevers to three different water contaminants (ethylbenzene, xylene, toluene) has been measured. Good agreement between the measured frequency shifts and theoretical predictions based on mass loading of the polymer-coated cantilever has been achieved. The detection of a concentration as low as 6 mg/L (corresponding to an added mass of 50 pg) of xylenes in water has been demonstrated. Furthermore, the sensing system has been used for the detection of PSA levels as low as 10 ng/mL in a sample fluid. The usage of the monolithic cantilever chip in a hand-held device for water quality monitoring or a dipstick-type disposable device for medical applications, such as body fluid analysis, can be envisaged.

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