

Rapid functionalization of cantilever array sensors by inkjet printing

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Abstract

The controlled deposition of functional layers is the key to converting nanomechanical cantilevers into chemical or biochemical sensors. Here, we introduce inkjet printing as a rapid and general method to coat cantilever arrays efficiently with various sensor layers. Self-assembled monolayers of alkanethiols were deposited on selected Au-coated cantilevers and rendered them sensitive to ion concentrations or pH in liquids. The detection of gene fragments was achieved with cantilever sensors coated with thiol-linked single-stranded DNA oligomers on Au. A selective etch protocol proved the uniformity of the monolayer coatings at a microscopic level. A chemical gas sensor was fabricated by printing thin layers of different polymers from dilute solutions onto cantilevers. The inkjet method is easy to use, faster and more versatile than coating via microcapillaries or the use of pipettes. In addition, it is scalable to large arrays and can coat arbitrary structures in non-contact.

1. Introduction

Initially, nanomechanical cantilevers were developed for atomic force microscopy, where they proved sensitive enough to allow imaging of surfaces with atomic resolution. Later, cantilevers were coated on one side with a sensor layer: having two chemically or physically distinct surfaces, they were used for sensor applications where the interaction with the medium causes a bending signal of the cantilevers. Their micrometre- or nanometre-scale size allows short response times, access to small-volume samples and parallel integration [1]. With thicknesses below one micrometre, cantilever sensors achieve extreme surface-to-volume ratios, that enables the conversion of surface stress induced by adsorbed molecules into mechanical bending. In addition, bilayer cantilevers are highly sensitive to changes of temperature and are also suitable for microcalorimetric measurements. For use in a dynamic mode, the cantilevers can be coated on both sides. Adsorbed

quantities lead to changes of mass, which translate into a change of resonance frequency when operated in an oscillating mode [2–4]. The readout of the bending of the cantilever is done optically by deflecting a laser beam via the cantilever onto a position-sensitive detector, or by means of an integrated piezoresistive element [5].

Today's microlithographic methods can achieve parallel fabrication of cantilever chips with well defined mechanical properties. For sensor applications, the cantilevers have to be coated with specific sensor layers that reversibly respond to different chemicals in solution or gas phase. In many applications, cantilevers are first coated with a thin gold layer on one side, which improves optical reflection for the optical readout and allows the adsorption of sensor layers using thiol chemistry. Examples for coatings are alkanethiols modified with different end groups to sense variations of pH or ions in liquids [6–8], or vapours from explosives in air [9]. Various polymers have been applied to detect pH changes [10] or vapours of solvents or water in air [3, 11–13]. Biosensors have been coated with single-strand DNA to hybridize its

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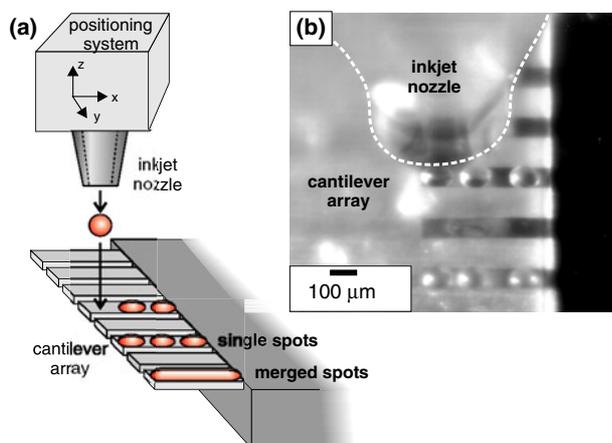


Figure 1. Inkjet printing of individual droplets onto a cantilever array (a) as a scheme and (b) as seen by a video camera. A positioning system allows accurate placement of single droplets onto selected cantilevers. When deposited with a small pitch, the droplets merge into a continuous layer covering the entire cantilever length. For demonstration, three droplets of water are deposited onto selected cantilevers. Owing to the oblique view of the camera, only the central cantilever is in focus.

complementary strand [14, 15], or antibodies to react with the corresponding antigen [16]. The strength of cantilever sensors is label-free detection and a sub-nanometre deflection sensitivity.

However, cantilevers are prone to side effects such as temperature variations, ionic strength of a bath or mechanical bending by hydrodynamic forces, which makes it difficult to clearly correlate the deflection signal of a single cantilever with specific binding events. The best strategy to rule out competitive effects is to use an array of cantilevers where unspecific or passivated cantilevers serve as internal references [17]. However, the challenge of such arrays is to functionalize the cantilevers individually. Usual concepts of microlithography using polymer resists fail because resist is difficult to apply to cantilevers and many sensor layers are not compatible with the solvents involved in resist processing. Some researchers have used focused ion beam etching to remove polymers selectively [12] or shadow masks to evaporate Au or other sensor layers selectively, but such processes are very time-consuming. Others have spray-coated or pipetted droplets of polymer solution onto cantilevers, but this turned out to be difficult to reproduce [3, 11]. For the formation of self-assembled monolayers (SAMs) from ethanolic or water-based solutions, immersion in small containers of liquid [6] or arrays of dimension-matched capillaries [15] has been proposed. However, it is challenging to avoid evaporation and retraction of the liquid from the cantilevers for typical immersion durations of 20 min.

A versatile method to deliver small amounts of liquids with good spatial accuracy is inkjet printing. The method is suitable for fabricating DNA microarrays [18] as well as for depositing SAMs or proteins [19]. Agilent Technologies (Palo Alto, CA) uses the technique even for DNA *in situ* synthesis on the substrate [20]. The uniformity of SAMs and DNA layers deposited by inkjet was verified recently using a selective etch method [21]. Inkjet deposition of polymers has also been demonstrated for printing functional electronic devices [22].

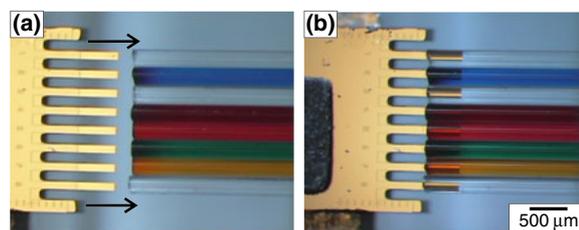


Figure 2. Immersion of a cantilever array into an array of glass microcapillaries. (a) The cantilever array was aligned with respect to the capillary array. The capillaries were filled with food colouring for demonstration purposes. (b) The thin part (500 μm long) of the cantilevers is inserted into the capillaries; thus the entire array is functionalized in parallel. No cross contamination was observed throughout the experiment.

In this paper we use inkjet printing to functionalize cantilever arrays. We demonstrate the successful deposition of self-assembled monolayers, polymer solutions and DNA samples, and verify the performance of cantilever sensor applications.

2. Materials and methods

2.1. Cantilevers

Cantilever arrays comprising eight identical silicon cantilevers at 250 μm pitch, having a length of 500 μm , a width of 100 μm and a thickness of 0.5–1 μm were provided by the micro- and nanomechanics group at the IBM Zurich Research Laboratory. Two different designs were used. The first type of chip (shown in figures 1 and 4) was fabricated by wet etch processes, and the cantilevers sit on tapered hinges. The second design (shown in figures 2, 5, 7 and 9) was fabricated by dry etch processes. The shape of the cantilevers has been extended in the solid body of the chip to prevent crosstalk during functionalization. Before further processing, the chips were cleaned in Piranha solution for 10 min (2 parts concentrated H_2SO_4 :1 part H_2O_2 ; *handle with care, reacts aggressively with organic compounds*). Cleaned arrays were coated with 1 nm of Ti (99.99%, Johnson Matthey) followed by 20 nm of Au (99.999%, Goodfellow) using an Edwards FL400 e-beam evaporator operating at a base pressure below 10^{-5} mbar at evaporation rates of 0.03–0.4 nm s^{-1} . Subsequent functionalization was performed within 2 days of Au deposition.

2.2. Compounds

All chemicals were of reagent grade or better and used as received unless indicated otherwise. Purified octadecanethiol (ODT) originated from Robinson Brothers Ltd (West Bromwich, UK). Hexadecanethiol (HDT) (Fluka, Buchs, Switzerland) was purified as described in [23]. Mercaptohexadecanoic acid (MHA) (Aldrich, Buchs, Switzerland) was dissolved in dichloromethane, filtered and dried. Mercaptoundecanoic acid (MUA) was synthesized as described in [24]. Thiol solutions were prepared in ethanol puriss. p. a. (absolute ACS reagent, Fluka) and stored in dark bottles.

The following solutions were used for pH-sensing experiments. A dilute 0.1 mM HCl served as a pH 4 sample

Table 1. Polymer solutions.

Abbreviation	Details	Solvent
CMC	Carboxymethylcellulose sodium salt 'low viscosity' (Fluka)	Water
PEG	Polyethylene glycol MW 6000 (Fluka)	Water
PEI	Polyethyleimine 'high MW' (Aldrich)	Water
PSS	Poly(sodium 4-styrene sulfonate) MW 70000 (Aldrich)	Water
PAAM	Poly(allylamine hydrochloride) MW ca 15000 (Aldrich)	Water
PVP	Poly(2-vinylpyridine) standard 64000 (Fluka)	Ethanol
PVA	Polyvinylalcohol 10–98 (Fluka)	DMSO
PMMA	Polymethyl methacrylate MW 15000 (Aldrich)	MIBK

solution. Prior to the experiments a pH of 3.8 was measured. A 0.1 mM NaOH solution was prepared freshly as pH 9 sample solution. The solution had a pH of 9.1, which was verified before starting the experiments.

Single-strand DNA (ssDNA) oligonucleotides were obtained from Microsynth (Balgach, Switzerland). Three different 12-mer probe sequences were used: B1 (ACA TTG TCG CAA), B2 (TGC TGT TTG AAG) and unspecific (ACA CAC ACA CAC); see also [15]. A thiol modification with a 5' HS(CH₂)₆ linker allowed a chemical bond to gold surfaces. 12-mer ssDNA samples of HPLC grade were dissolved in water and extracted by three cycles of ethyl acetate washing, followed by centrifugation and phase separation to carefully remove dithiothreitol, a compound that had been added to the modified DNA to preserve the thiol linker. For the coating of cantilevers, 40 μM of thiolated ssDNA sample solutions was prepared in 50 mM triethylammonium acetate (TEAA) buffer (Fluka). A solution of B1C (TTG CGA CAA TGT) non-thiolated target DNA complementary to the B1 compound was prepared in sodium saline citrate (SSC) buffer concentrate (Fluka) diluted 2:1 in deionized (DI) water. A concentration of 100 nM was used for sensor experiments.

Polymer solutions were prepared with a concentration of 5 mg ml⁻¹ in water, ethanol, dimethylsulfoxide (DMSO) or methylisobutylketone (MIBK); see table 1. In order to achieve good wetting and adhesion on the Au-coated cantilevers, the surfaces were primed with a SAM of MUA or MHA by immersion in a 1 mM solution for 15 min followed by a brief rinse in ethanol.

2.3. Inkjet printing

An MD-P-705-L inkjet dispensing system (Microdrop, Norderstedt, Germany) was equipped with a three-axis micropositioning system having an accuracy of 10 μm and piezo-driven autopipettes AD-K-501 with 70 μm nozzle diameters. The piezo-pipettes were filled from the front side, allowing the use of small sample volumes of 5–25 μl. The pipettes can be cleaned using common agents, as their core is made of glass. In addition, the piezo-element enables cleaning by ultrasonic agitation. A stroboscopic camera system provided visual control to adjust piezo voltages and

pulse durations for reliable droplet ejection and to avoid satellite drops. Single droplets with diameters in air of 60–80 μm corresponding to volumes of 0.1–0.3 nl were ejected on demand. Figure 1 shows a schematic overview and illustrates how water droplets were deposited onto Si cantilevers. When droplets were spotted with pitches smaller than 0.1 mm, they merged and formed continuous films. The vertical separation between the nozzle and the sample was typically 0.4 mm.

2.4. Capillary functionalization

For control experiments, the cantilevers of an array have been immersed into the open ends of a microcapillary array filled with various coating solutions for functionalization, as shown in figure 2. The glass capillaries (Garner Glass Company, CA, USA) had an outer diameter of 0.25 mm, an inner diameter of 0.18 mm and a length of 75 mm. The design of the system allowed immersion times of up to 2 h because liquid fluxes from reservoirs at the back end of the glass capillaries compensated for evaporation losses at the front end.

2.5. Selective etch

The selective etch bath was composed of 20 mM Fe(NO₃)₃ (Fluka) and 30 mM thiourea (Fluka) in DI water [21, 25]. Cantilever arrays were immersed in the etch bath without stirring until the non-treated (bare surface) Au was clearly removed. Etching times were 10–30 min. The samples were cleaned by dipping into DI water and ethanol, and finally inspected with optical microscopy or scanning electron microscopy (SEM) (Leo 1550, LEO, Oberkochen, Germany).

2.6. Sensor instrument

The mechanical response of the cantilever sensors was detected using an optical readout system shown in figure 3. The set-up comprised an array of vertical cavity surface-emitting laser (VCSEL) diodes (Avalon, Zurich, Switzerland) with an optical power of up to 2 mW and a wavelength of 760 nm, focused onto the cantilevers. The reflected beam was detected by a position-sensitive diode. The functionalized cantilever array was placed in a sensor chamber suitable for liquid- or gas-phase sensing. The set-up allowed quasi-simultaneous readout of up to eight cantilevers by time-multiplexing the lasers in 80–400 ms intervals.

The sensor chamber for liquids had a volume of ~80 μl and was supplied via Teflon tubes (Supelco, Bellefonte, PA) using a syringe pump (Genie, Kent Scientific, Torrington, CT) and a ten-position valve selector (Rheodyne, Rohnert Park, CA) connected to different sample solutions. The temperature, recorded using a type K thermal sensor in close proximity to the sensor chamber, was maintained at 23 ± 0.2 °C.

The gas sensor chamber had a volume of 300 μl. Analyte vapour was generated in a 2 ml vial into which about 0.5 μl of liquid analyte was injected through a septum using a needle syringe. The vapour from this liquid analyte was pumped through Teflon tubes to the sensor chamber. Nitrogen gas was used as carrier gas. Using mass flow controllers (Bronkhorst HI-TEC, AK Ruurlo, The Netherlands), the flow was adjusted to 24 ml min⁻¹. Valves were used to switch between analyte gas and pure nitrogen [3].

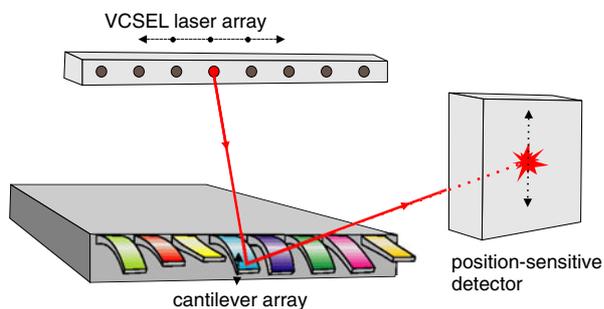


Figure 3. Optical readout of a cantilever array sensor by reflection of a laser beam. If the cantilever bends, the laser spot on the position-sensitive detector moves.

3. Results and discussion

3.1. Alkanethiol functionalization

Alkanethiols are known to form SAMs on Au surfaces [26]. Such molecules consist of a sulfur group that covalently binds to the Au, and an alkyl spacer with a functional head group. Ideally, the surface properties, such as wettability, charge and reactivity, are governed by the chemistry of this functional group. Densely packed monolayers are achieved by immersing gold-coated surfaces into millimolar solutions for a few minutes. It was recently shown that inkjet printing can produce uniform spots of SAMs, although the tiny sub-nanolitre droplets of ethanolic solutions delivered onto the substrates evaporated in less than 1 s [21].

The condensation of microdroplets is a simple test to characterize the wettability of different SAMs [19, 27]. With the inkjet system we prepared eight cantilevers with alternating hydrophobic and hydrophilic monolayers of ODT and MUA, respectively. Six droplets of 1 mM solutions were printed onto each cantilever. Excess thiol was removed in an ethanol bath. Then, the chip was placed on a Peltier element and cooled until ambient water condensed on the surface (figure 4). The difference in wettability of the monolayers appeared clearly in this condensation experiment. The untreated Au on the body of the chip shows intermediate-size water droplets, whereas the droplets are significantly larger on the hydrophilic MUA monolayer and much smaller on the hydrophobic ODT film.

However, detailed contact angle measurements are difficult to carry out on microcantilevers. The same is true for ellipsometry and most other surface characterization techniques. In order to verify the presence of SAMs, we applied a wet etching method [21] that efficiently removes the Au layer except where it is protected by a SAM. The residual Au films on etched samples were inspected by SEM or optical microscopy. This selective etch diagnostics was applied to optimize the deposition parameters on a test cantilever array as shown in figure 5: cantilever 1 was temporarily coated by a layer of CMC as a control to protect the Au. Cantilevers 2–6 were coated by inkjet with 2–40 droplets of 4 mM HDT. After that, cantilever 8 was immersed into 4 mM HDT using a microcapillary. Some HDT crystallites were observed by optical microscopy on the coated cantilevers. After brief immersion baths, first in ethanol to remove excess HDT, then in water to remove the CMC layer, the array was put into a selective etch bath for 30 min. The SEM inspection of the array

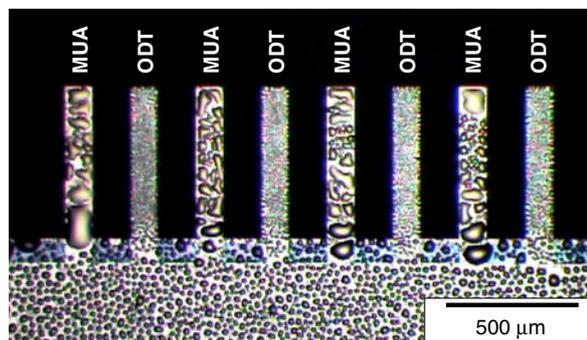


Figure 4. Optical microscope view of microdroplet condensation on a cantilever array. The cantilevers were inkjet coated with alternating monolayers of ODT (hydrophobic) and MUA (hydrophilic) as indicated. Cooling of the chip below the dew point caused droplets of water to condense on the surface. Their difference in size and density reflects different wettability properties of the cantilevers and of the untreated Au surface on the chip.

in figure 5 showed complete removal of the Au on cantilever 1, whereas defect-free Au films (bright regions) were preserved on all of the SAM-coated cantilevers, which indicated that good quality SAMs had been achieved. In addition, cantilever 7 also appeared to be protected by a monolayer. Here, we suggest that a SAM has been formed through vapour transport from neighbouring cantilevers. Indeed, air is not a good barrier for volatile thiols. For example HDT has a vapour pressure at room temperature of 4×10^{-3} Pa [23], which is not negligible, as independent experiments showed that a complete monolayer of HDT can be formed from the vapour phase within 30 min [28]. A diffusion constant of about $D = 0.04 \text{ cm}^2 \text{ s}^{-1}$ in air allows transport over a diffusion length of 2 mm within 1 s, which is approximately the dimension of the entire cantilever chip [29]. Such cross contamination can be minimized by preparing cantilever arrays starting with the least volatile thiols, avoiding material excess, or protecting adjacent cantilevers temporarily with resist layers such as CMC.

Finally, the performance of cantilever sensors coated via different techniques was directly compared on one array. Two cantilevers were coated by inkjet with 30 droplets of 4 mM MHA. This quantity was sufficient to form a good monolayer as indicated by the etch studies. The same amount of 4 mM HDT was applied to two other cantilevers. The remaining cantilevers were immersed for 5 min into an array of microcapillaries filled with 4 mM MHA or HDT, respectively. Then, the array was cleaned of excess thiols by immersion in ethanol for 3 min. It was important to perform the entire coating procedure within a few minutes in order to minimize cross contamination of untreated cantilevers. MHA was deposited as the first compound because—due to its polar group—it is significantly less volatile than HDT.

The cantilever array was equilibrated overnight in the sensor chamber in pH 4 sample solution. The chamber was alternately flushed with 5 ml of pH 4 and pH 9 solutions, respectively, at a rate of 1 ml min^{-1} . The deflection responses of the cantilevers are shown in figure 6. Traces were recorded of two HDT-coated and two MUA-coated cantilevers. One of each type had been prepared by inkjet, the other one by immersion into capillaries. Independent of the coating method, cantilevers with the same monolayers showed almost identical

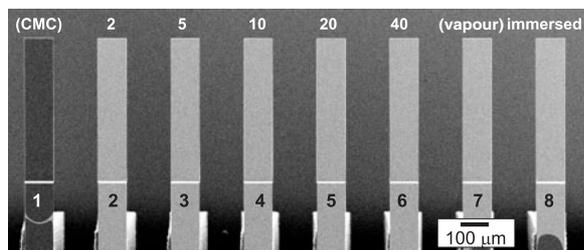


Figure 5. SEM micrograph of a functionalized cantilever array after a selective etch of gold. The array had been coated with HDT using inkjet (levers 2–6) or capillary immersion (cantilever 8). The amount of droplets (2–40) printed by inkjet is indicated above the cantilevers. Cantilever 7 had been coated indirectly with HDT by vapour transport from neighbouring cantilevers. Cantilever 1 served as a control. It had been temporarily protected by a layer of CMC to prevent contamination of the surface with thiols that potentially evaporated from neighbouring cantilevers. The gold was completely removed here.

deflection responses throughout the experimental sequence. The initial deflections were set to zero. During the periods of injection that are shaded in figure 6, the pumping mechanism caused oscillations in the cantilever deflections. The injected liquid appeared to be $0.2\text{ }^{\circ}\text{C}$ warmer than the sensor unit. As soon as the flow was stopped, the temperature in the cell recovered, which produced a bimetallic response of 50 nm. A steady negative drift was observed during the exposure to pH 4 solution. Upon injection of pH 9 solution, the cantilevers showed a quick common response, which we attributed to the non-coated Si back side that is identical for all cantilevers. After 30 s, the COOH-terminated cantilevers (red and green traces) inverted this trend and deflected in the negative direction for about 3 min until a maximum differential signal of about 360 nm was reached, as shown in figure 6(b). This cycle was repeated.

Our experiments prove that we could achieve the same functionality with SAMs coated by inkjet and by capillary immersion. However, compounds that are more volatile than HDT should be avoided in order to minimize cross contamination via vapour transport in air.

3.2. DNA oligomer sensors

The 12-mer DNA compounds have more than 20 times the molecular weight of MUA or HDT, hence they are significantly less volatile and do not require precautions concerning vapour transport. But as they are less mobile, they require longer reaction times to chemisorb with their reactive thiol group on the gold surface and to assemble into a dense monolayer. At relative humidities of 20–50% in air, the droplets printed by inkjet evaporated from the substrate within a few seconds. Despite such a short reaction time, the selective etch method indicated a dense monolayer formation [21]. Again, the etch diagnostics was applied to optimize the inkjet printing conditions for the formation of DNA monolayers on cantilevers. The bright regions in figure 7 show exactly where monolayers have protected the gold on cantilevers that had been treated with different quantities of DNA solution. Cantilevers 1 and 7 had not been coated at all and served as a reference. The gold was completely removed there, as evidenced by their dark colour. As expected, there was no

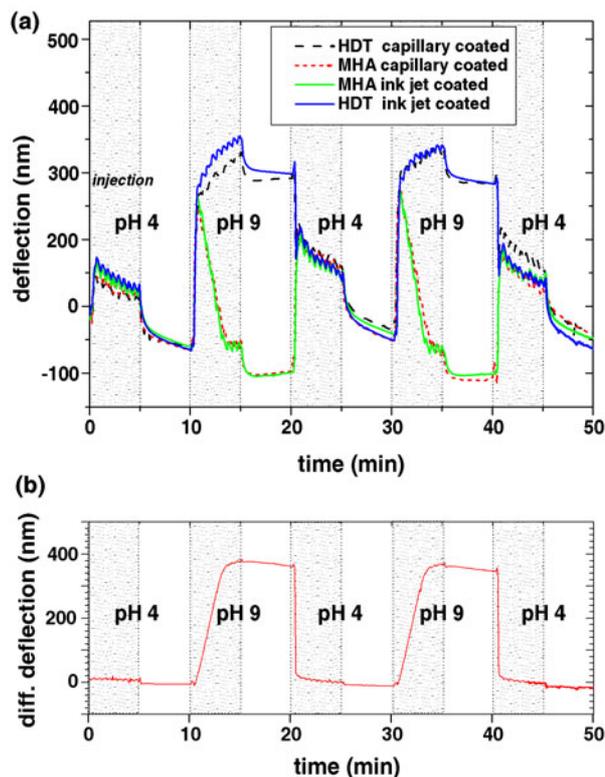


Figure 6. Deflection responses of cantilever sensors on the injection of dilute HCl (pH 4) and dilute NaOH (pH 9) solutions. (a) The HDT-coated cantilevers show the same response during the experimental flow, independent of their manner of preparation, by capillary immersion (black dashes) or inkjet (blue trace). The MHA capillary-coated (red dashes) and inkjet-coated (green line) cantilevers also behave equally. Positive deflection corresponds to a compressive stress of the monolayer sides. The injection periods are shaded. (b) The differential deflection (HDT signal – MHA signal) provides a clear sensor signal.

effect of cross contamination from neighbouring cantilevers. On cantilevers 2–6, a number of droplets increasing from 5 to 64 had been printed as indicated in the figure. Five or ten droplets did not completely cover the cantilever, whereas 16 or more droplets, distributed over the entire cantilever length, formed a continuous and well-protecting monolayer. For direct comparison, cantilever 8 has been traditionally coated by immersion for 20 min into a microcapillary filled with DNA solution. However, the monolayer shows some imperfection: at the cantilever's end the gold has been partially attacked by the etch. These etch results prove that cantilevers can be uniformly covered with dense DNA layers by inkjet printing.

The functionality of ssDNA-coated cantilevers was verified in a sensor experiment. 500 nm thick cantilevers were coated with B1, B2 and unspecific DNA probes. After several hours of equilibration in SSC buffer, the chip was exposed to an injection of SSC and 100 nM B1C solutions. Figure 8 shows the differential signal of the B1 and B2 cantilevers relative to the unspecific-coated cantilever. The data clearly demonstrate a specific response of the B1-coated cantilever upon injection of the complementary B1C target. The deflection is attributed to a compressive surface stress due to a hybridization reaction. The reaction was reproducible as the target could be washed off successfully by cycles of buffer solution.

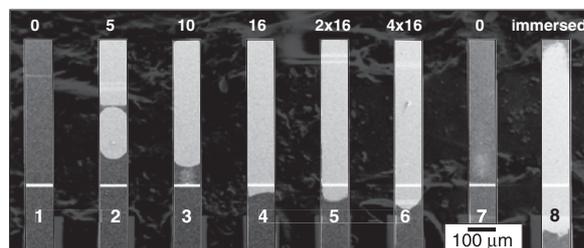


Figure 7. Etch diagnostics of a cantilever array. Different quantities ($0 \dots 4 \times 16$ droplets, as indicated) have been printed by inkjet onto adjacent cantilevers in order to optimize the deposition parameters. The SEM clearly shows the remaining gold (bright areas) that has been protected by ssDNA oligomer films. Cantilever 8 has been coated using capillary immersion, but the partial removal of gold at this cantilever's end indicated local imperfection.

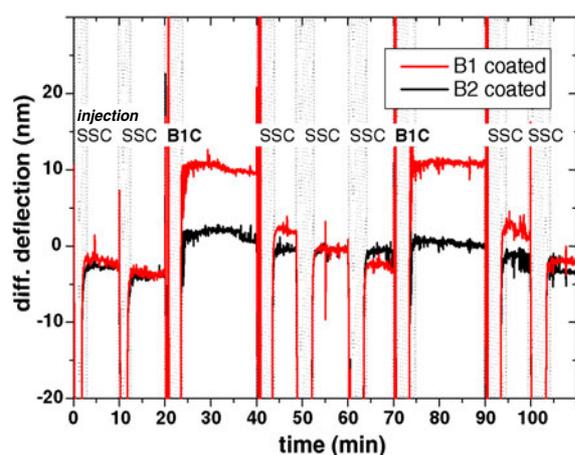


Figure 8. Experimental data of B1- and B2-coated cantilevers on the injections of SSC buffer and 100 nM B1C in SSC buffer. The B1-coated cantilever shows a clear reproducible signal upon the injection of its complementary B1C target. During the injection (shaded regions) the cantilevers experienced strong bending from hydrodynamic forces. Baseline subtraction compensated for linear drift.

These experiments prove that functional DNA probes can be printed by inkjet within a few seconds. The coating of an entire chip with different probe layers requires only 1–5 min, whereas the immersion in microcapillaries takes 15–30 min. In an additional experiment where both sides of the cantilevers were coated with gold, the etch test showed that DNA can be printed onto one side of the cantilevers without contaminating the backside. This is especially important when cantilevers are to be coated with proteins or other layers that would adsorb unspecifically on both sides when immersed. In [19] it was shown that inkjet printing is also suitable for the deposition of proteins. If necessary, the operation of the inkjet system in a controlled humid environment can extend the lifetimes of deposited droplets to allow longer adsorption times. A humid environment is also beneficial for maintaining a hydration of adsorbed layers, which is essential for example when proteins are involved.

3.3. Polymer coating

As opposed to SAM systems, polymers do not selectively assemble on Au surfaces and the thickness is not self-limiting.

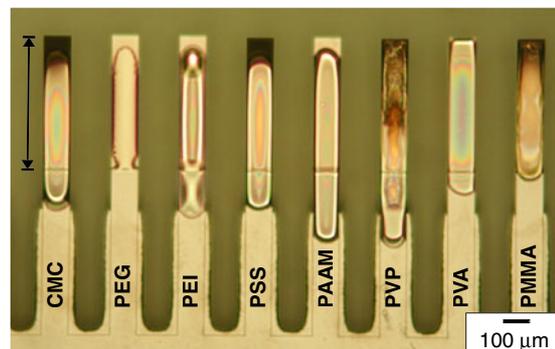


Figure 9. Optical micrograph of cantilevers coated with different polymers by inkjet printing of dilute solutions. The acronyms of the polymers are explained in table 1. The arrow indicates the thin part of the cantilevers.

Inkjet is a preferred method to place tiny volumes of polymer solutions accurately onto microcantilevers. We chose eight different polymers, which had different polar groups and potential affinities to natural flavour compounds. The coated cantilever array is shown in figure 9. Most of the polymer films appeared to be thicker at the periphery and thinner in the centre of the deposit due to drying effects [30]. Coloured interference fringes reflect the variation of film thickness. The amount of polymer had been adjusted between 0.4 and 1.4 μm nominal thickness. Accurate alignment was important to prevent the migration of droplets to the backside of a cantilever, which was verified by optical microscopy.

In a basic test experiment, the cantilever array in the sensor chamber was exposed to vapours of water and ethanol, figure 10. The initial deflections were set to zero when the valves opened at time zero, and after a delay of a few seconds (partially due to the connector tubing), the cantilevers bent with different amplitudes and response times. The response pattern to water vapour, figure 10(a), where all of the cantilevers show a pronounced action, is clearly different from that of exposure to ethanol, figure 10(b), where only the cantilevers coated with PEG, PEI, PVP and PMMA showed significant bending. The deflections are of the same order of magnitude as in the experiments of Baller *et al* [11], although we used polymer layers about a factor of five thinner. After each measurement cycle, the chamber was purged with nitrogen gas for a few minutes. A principal component analysis [11] was carried out using the deflections at 10, 30 and 50 s. The results show in figure 10(c) the extracted data of each cycle projected as a single spot into a two-dimensional chart of principal components. The data points of water (blue) and ethanol (yellow) cluster within small regions in the chart, demonstrating reproducible sensor measurements. In addition to the pure compounds, we also measured a 1:1 mixture of ethanol and water (green). It was clearly distinguished from the pure compounds.

4. Conclusion

This study demonstrates that inkjet printing is a rapid and general method to functionalize nanomechanical cantilevers with various sensor layers for liquid and gas sensing applications. The combination of drop-on-demand technology

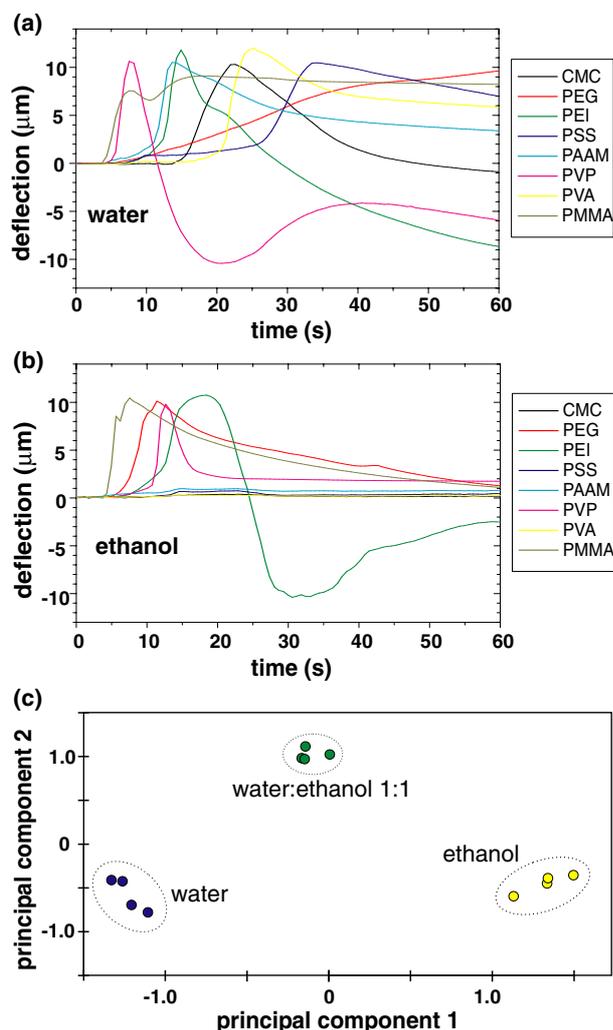


Figure 10. Experimental data of the polymer-coated cantilever array sensing vapours of water and ethanol. The deflection traces of (a) water and (b) ethanol show specific deflections for the cantilevers coated with specific polymers. The label codes of the polymers are explained in table 1. A principal component analysis (c) projects the complicated responses on two coordinates. The characteristic responses to vapours of water, ethanol and a mixture of water and ethanol are distinguished reproducibly.

with an accurate positioning and alignment system allowed the non-contact coating of microcantilevers within a few seconds with reproducible quantities of sub-nanolitre solutions. Owing to its speed and versatility, the inkjet can replace time-consuming coating in microcapillaries and the use of pipettes. In addition, inkjet printing has the advantage that only one side of a cantilever can be coated without contamination of the backside. It is suitable for automation, and multiple nozzles can be operated in parallel for large-scale applications.

By providing an efficient method for functionalization, the outcome of this work should help to establish the technology of nanomechanical cantilever sensors for chemical, biochemical and medical applications. In general, inkjet printing is not restricted to cantilever bars: coatings can be patterned on any flat assays or applied to arbitrarily shaped sensors [13, 31] or to structures that are difficult to functionalize with other methods.

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