Scanning probe arrays for life sciences and nanobiology applications

Laure Aeschimann a,*, André Meister b, Terunobu Akiyama a, Benjamin W. Chui a, Philippe Niedermann b, Harry Heinzelmann b, Nico F. De Rooij a, Urs Staufer a, Peter Vettiger a,b

a Institute of Microtechnology, University of Neuchâtel, Rue Jaquet-Droz 1, 2007 Neuchâtel, Switzerland
b Swiss Center for Electronics and Microtechnology CSEM SA, Rue Jaquet-Droz 1, 2007 Neuchâtel, Switzerland

Abstract

Various 1D and 2D scanning probe arrays with integrated piezoresistive sensors have been developed in view of nanobiology and life science applications. For such applications, the experiments need usually to be performed under liquid conditions. The probe arrays have been fabricated by using silicon micromachining techniques and SOI wafers. For biological applications, when the cantilevers are immersed in electrically conductive buffer environments, the piezoresistive sensing elements have to be protected. For this purpose, the sensors were coated with a 50 nm thick silicon nitride film. The realized piezoresistive cantilevers had low spring constants between 0.1 and 0.5 N/m, well suited for biological applications. Parallel imaging using a 1 × 2 probe array was demonstrated by simultaneous imaging of a test sample in buffer solution.

Keywords: Scanning probe array; Life science; Nanobiology; Piezoresistive sensing; Parallel imaging; AFM

1. Introduction

The scanning probe microscope is a powerful instrument for measuring surface properties with nanoscale resolution. Especially, its ability to operate in liquid environments has led to numerous biological imaging and force measurement applications. However, its slow scan speed considerably limits the throughput. It has been shown that this can be overcome by parallel operation of large probe arrays [1,2]. Integrated functionality such as the nanometer scale detection of cantilever deflection will greatly simplify the operation of large 2D probes.

The main objective of this project is to design, fabricate and operate 2D probe arrays with integrated functionality suited for life sciences and nanobiology applications. Investigations on biological samples need to be performed in their natural environment, which usually means under liquid conditions. Up to now, little work was done in liquids using other detection schemes than optical read-out. The liquid environment imposes stringent requirements on sensors performances and life-time. We propose to adapt piezoresistive strain gauges [3] for operation in liquids, avoiding electro-chemical reactions and leakage currents that could occur at the sensors [4]. This approach could be applied for imaging of biological samples or single molecule force spectroscopy experiments, e.g. to investigate the binding forces of proteins embedded in cell membranes [5].

Our approach consisted of passivating piezoresistive probes with a silicon nitride film. First experiments performed with silicon nitride coated single piezoresistive cantilevers had shown that operating in water was possible. Compared to an uncoated lever operated in air, no degradation, i.e. a change in sensitivity or different noise characteristics was measured. Moreover, the output signal of the piezoresistor immersed in a buffer solution proofed to be stable over several hours. These experiments clearly demonstrated that the isolation layer sufficiently sealed the piezoresistive sensor against its liquid environment.

* Corresponding author. Tel.: +41 32 720 5619; fax: +41 32 720 5711.
E-mail address: laure.aeschimann@unine.ch (L. Aeschimann).
Based on these results obtained on single levers, various probe arrays were designed and corresponding fabrication processes were established.

2. Fabrication

A silicon-on-insulator (SOI) wafer with a 10 μm n-type silicon film and a 0.5 μm buried oxide layer on a 345 μm thick silicon handle wafer was selected as substrate. Anisotropic silicon etching in KOH was used to undercut circular oxide masks to form tips in the device layer. This step also determined the cantilever thickness. After an oxide sharpening of the tips, the contact pads were heavily doped by boron diffusion. Then, an 80 nm thick oxide layer was thermally grown on the wafer. To form the piezoresistors, a resist pattern was used to mask boron ion implantation at 40 keV, executed with a dose of $2.5 \times 10^{14}$ cm$^{-2}$, followed by rapid thermal annealing at 1000 °C. These parameters resulted in a shallow implant near the surface of the cantilever, increasing deflection sensitivity [6,7] (Fig. 1a). As passivation layer for the piezoresistors a 50 nm thin film of LPCVD silicon nitride was deposited and patterned (Fig. 1b). Contacts for the resistors and wiring were realized by evaporation and patterning of a 1 μm thick aluminum film. The metal lines were then passivated in a patterned hard-baked photoresist (Shipley AZ4562) layer (Fig. 1c). This step was important since the metal wires had to be protected similarly to the piezoresistors for measurements in liquids. The cantilevers were then formed from the topside by silicon dry etching (Fig. 1d) and released from the back using a deep reactive ion etch, which stopped on the buried oxide. In a last step the buried oxide was removed by wet etching in buffered hydrofluoric acid (Fig. 1e).

In addition to the standard cantilevers, honeycomb-like patterned cantilever designs have been investigated. The goal was to determine whether the sieve-like cantilever body would reduce the damping during liquid operation. For the fabrication of these cantilevers the starting substrate was again a SOI wafer. The device layer was 15 μm, the buried oxide layer 1 μm and the handle wafer 380 μm thick. The sieve-like structures of the cantilever body were realized by silicon dry etching just after tip formation. The other steps of the fabrication procedure were the same as for the plain levers.

3. Results and discussion

A scanning electron microscope image of a fabricated 4 × 4 probe array is shown in Fig. 2a. Beneath each cantilever, a reference resistor was implemented, which made it possible to compensate background noise such as thermal drift. Fabricated cantilevers were about 135 μm long, 30 μm wide and 1–1.5 μm thick, which led to low stiffness with typically measured spring constants of 0.1–0.5 N/m, as required for soft biological samples. The tips were 5 μm high and the apex radius was about 10 nm as measured from SEM images (Fig. 2b).

The piezoresistive sensitivity was determined by measuring the resistance change as a function of the cantilever bending. For this purpose, the probe was mounted in a DI Multimode AFM and force distance curves on a hard substrate were performed. The response was measured with a full Wheatstone bridge circuit and a home-built amplifier system. The bridge voltage supply was 6 V and the amplifier gain was 1000. A deflection sensitivity of
$2 \times 10^{-7} \Delta R/R$ per Å was measured. This value is in agreement with data published earlier, especially for soft cantilevers [6,8].

A 1 × 2 probe array was used for imaging on a test sample in order to demonstrate functionality. For that purpose a scratched silicon surface was imaged in constant height mode in a buffer solution (PBS, pH 7.4). The simultaneous recorded images are depicted in Fig. 3. The scan range was such that the two pictures were overlapping. This is pointed out in Fig. 3 by the dotted line.

Also the honeycomb-style piezoresistive cantilevers were investigated for liquid operation. Fig. 4 shows a SEM image of a 1 × 2 array of such cantilevers. The piezoresistor was incorporated into the short flat section at the fixed end of the cantilever. Only the cantilever on the right side in Fig. 4 had a hollow body. The one on the left side was solid and used as control lever. Basic performance of these cantilevers was very similar to that of the conventional levers. But, measurements acquired in water showed that there was no observable advantage in Q factor or resonant frequency when using the honeycomb cantilever as compared to using the solid probe.

4. Summary and conclusions

1D and 2D scanning probe arrays with integrated piezoresistive read-out were designed and successfully fabricated. The stiffness of the cantilevers was low, between 0.1 and 0.5 N/m as necessary for the imaging of soft biological samples. The piezoresistive sensors were passivated with a thin silicon nitride film in order to protect them during operation in liquid environments. Long-term stability of the passivation layer was demonstrated by immersing the probes in a buffer solution and measuring the output signal of the sensors over few hours.

The performance of conventional and honeycomb-style cantilevers was compared. It was found that the use of this special design confers no improvement in terms of Q factor and resonant frequency. A better understanding of the hydrodynamic behavior of such cantilevers should be obtained by theoretical modeling, and optimization of the design could be achieved.

For the conventional probes, parallel imaging capability in buffer solution was demonstrated by scanning a test sample with two cantilevers in parallel. These results are very
promising in view of future 2D probe array experiments for biological and life sciences applications.

Acknowledgements

This project is financed by the Board of the Swiss Federal Institutes of Technology through the program TOPNANO21. We thank the technical staff of the CSEM/IMT common lab COMLAB for their support.

References


