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SiO_2 Microcantilevers Designed for Biosensing: Experiments and Simulations

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Abstract. A cantilever array with dimensions of the beams 150×80×1.2 μm has been fabricated. Three different biochemical compounds were deposited and the cantilever was investigated using scanning electron microscopy (SEM). Simulations using CoventorWare have been made and stresses have been calculated between 12 and 60.4 MPa.

Keywords: cantilever, biosensing, transducers, stress, simulations, MEMS.

1. Introduction

The high market demand for commercial biosensors recently provoke a great interest in investigation and development of associated technologies. A huge variety of biosensors already exists starting from the canary in cage up to the blood glucose biosensor. Generally, a biosensor consists of 3 parts: the sensitive biological element, the detector element, and transducer in between, which associates both elements and converts the physical change, accompanying the reaction of the biological element into a measurable parameter.

Cantilevers are widely used in numerous applications, from the big ones employed in buildings and aircrafts wings to the miniaturized ones in the microelectromechanical systems (MEMS) where cantilevers are the most ubiquitous structures. Besides

Atomic Force Microscopy (AFM), where cantilever is essential, application of the cantilever transducers in medical diagnostics attracts interest of a large number of research groups [1–3]. Some of advantages of the cantilevers compared with conventional sensors are: possibility of performing label free molecular recognition with high resolution [2], real time measurements [4], increasing of speed, higher sensitivity [5], and better reproducibility due to technological processes which allows reproducible geometrical shapes.

Chemomechanical actuation of a microcantilever beam induced by biomolecular binding such as DNA hybridization and antibody-antigen binding is an important principle useful in biosensing applications[6]. Microcantilevers are highly sensitive to mechanical stress, which is largely caused by surface tension of layers deposited onto a cantilever. A thin bio-molecular layer, with the potential to bind biochemical analytes to the cantilever surface, will change the deflection of the cantilever in the presence of a biochemical specimen. The cantilever deflects upwards or downwards. As the magnitude of the forces involved is very small, increasing the sensitivity of the microcantilever beams involved is a priority [5].

This article presents the fabrication of an array of cantilever beams. Three different biochemical compounds were deposited on the cantilevers. The deflections of the beams as well as their surfaces were investigated by Scanning Electron Microscopy (SEM) as well as Atomic Force Microscopy (AFM). Then, the stress on the surface, caused by deposited compounds was estimated using the CoventorWare simulation program. The data give some insight about parameters of the deposited layer and allow to reach some conclusions about sensitivity and reability of manufactured cantilevers.

2. Experiments

2.1. Fabrication

A SiO_2 cantilever beam test array was designed. The microstructures were manufactured on Si <100> wafers using a technological process based on silicon micromachining techniques. The detailed fabrication process is shown in Fig. 1. The substrates were preliminary cleaned with 3-Cl-ethylen and dried for 30 min at 90°C. The first step was to grow a thick SiO_2 layer (1 μm) by means of dry thermal deposition (Fig. 1, a)). Then SiO_2 was patterned by polyphotography and etched (Fig. 1, b)). After that, front side anisotropic etching of Si in KOH 25% solution at 80°C was performed forming, in this way, SiO_2 cantilevers (Fig. 1, c)). Finally, 0.02 μm Cr and 0.2 μm Au layers were evaporated.

The cantilevers in array are 1.2 μm thick, 150 μm long and 80 μm wide. The distance in between is 140 μm. The cantilevers were coated with Cr/Au in order to allow the thiol group (-SH) of the deposited biocomponents to form an ordered monolayer which will anchor the receptor. This metallisation is done only on the upper side of the cantilever in order to maximize the differential change in the surface stress.

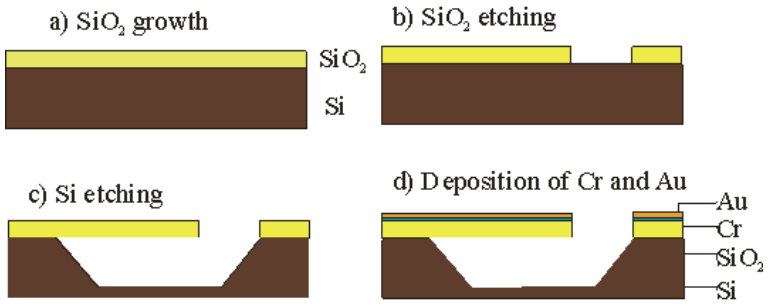


Fig. 1. Schematic view of the fabrication process.

2.2. Self assembling monolayers immobilization

The specificity and sensitivity of a cantilever based biosensor is given by the proper coating of its surface with a uniform and efficient layer of highly specific receptors. One technique to anchor a stable layer of molecules onto a solid surface is based on self assembling monolayers (SAMs) like thiols. On a specific substrate, such molecules, generally, form a well ordered, densely packed and strongly bond monolayer which can be then used as an intermediate layer to anchor the true receptor (e.g. antibody) [7].

The purpose of the experiments was the realization of a SAM on gold supports based on the affinity of the thiolic molecules for gold surfaces and then, its detection by the cantilever deflection.

For the thiolic compounds immobilization on gold supports it is necessary to perform a preliminary thorough surface cleaning. For this cleaning, it was employed a protocol based on highly oxidative Piranha solution (3:1 sulfuric acid concentrate: hydrogen peroxide 30%). The gold surfaces were kept for one hour in freshly prepared Piranha solution, then thoroughly washed with ultra pure water (MiliQ, 18 Mohm) and immersed in freshly prepared solution of enzyme, the thiolic compounds or ultra pure water (for the blank). Before measurements all the samples were taken out from the solutions and were left to dry in air for one hour.

There were used two thiolic compounds: L-cysteine (L-Cys, (L)-2-amino-3-mercaptopropionic acid, FW 121, Fluka) and L-glutathione reduced (GSH, 2-amino-5-[1-(carboxymethylcarbamoyl)-2-sulfanyl-ethyl]amino-5-oxo-pentanoic acid, FW 307, Merk) and one enzyme: Invertase (EC 3.2.1.26, Sigma).

The proteins, generally, have the tendency to non-specifically adsorb on different solid surfaces including gold. Based on these interactions, the clean surface was modified with invertase. Invertase is used as a model compound that may be adsorbed on the cantilever. Invertase has a very large molecule in comparison with the thiols such that the signal of the cantilever deformation should be higher than in the case of the thiols.

2.3. Characterization

The images were acquired with a home-built AFM operated in contact mode as well as Scanning electron microscope TESCAN VEGA (SEM) with working param-

eters: working mode resolution, accelerating voltage 10 kV–30 kV, working distance 10 mm–40 mm, detector: secondary electron.

Scanning speed of AFM varied between 1 and 2 Hz (lines/s) and the set point was kept to minimum (zero). The AFM sensors (microcantilevers) from Park ScientificTM were triangular with k = 0.03 N/m and nominal tip radius $\sim$ 20 nm.

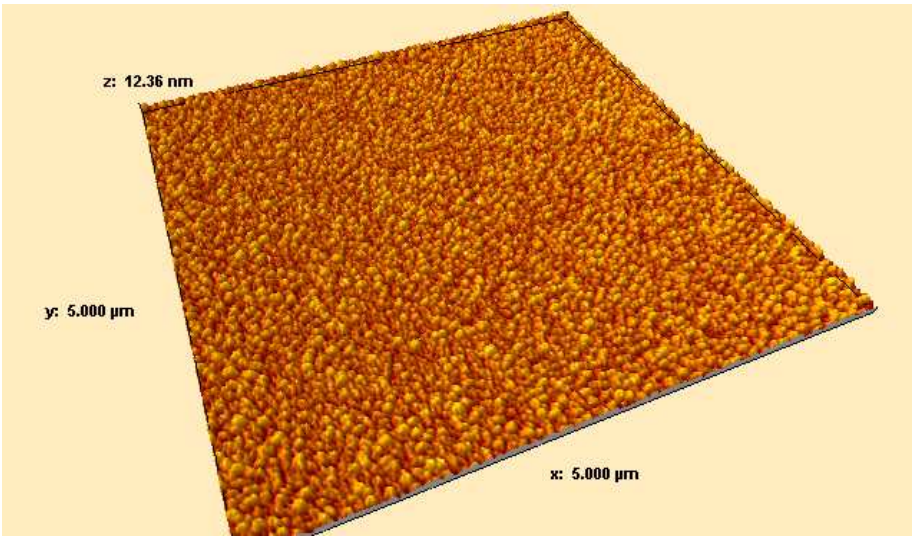
The mean deflection of the cantilevers has been estimated with an accuracy of $\sim$1 μm using SEM pictures made in the same plane.

Fig. 2a shows an AFM picture of bare Au surface (used as reference), exhibiting a very regular structure with uniform granularity. In Fig. 2b one can see the same substrate, covered with L-Cys and respectively in Fig. 2c with electrochemically deposited GSH. For the all three images the scan size was 5×5 microns. The surface morphology changes could be easily perceived in Fig. 2b and 2c in comparison with Fig. 2a.

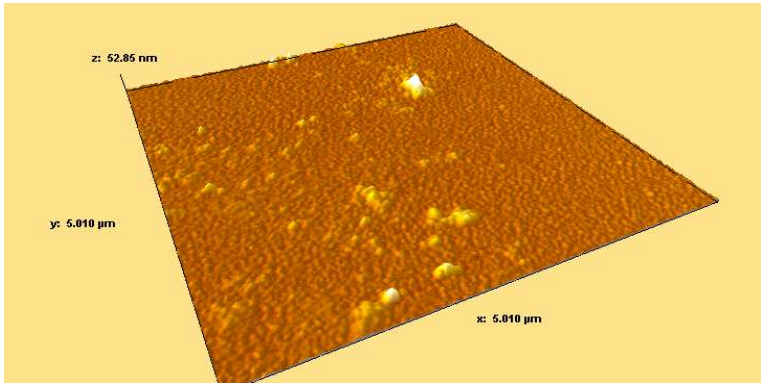
For a more quantitative assessment, surface roughness was estimated for all three samples from the AFM data with similar scan area (5×5 microns). The rms roughness value increases from 1.52 nm for pure Au substrate to 2.39 nm for L-cys and 8.00 nm to GSH, which represents a clear indication of some deposition on the Au surface.

Figure 3 shows the SEM picture of a cantilever array used as a blank. The sample was cleaned at the same conditions as mentioned above and stored in a water container. A slight initial upward deflection of the cantilevers up to 2 μm, probably due to stress caused from the Cr-Au holding has been seen.

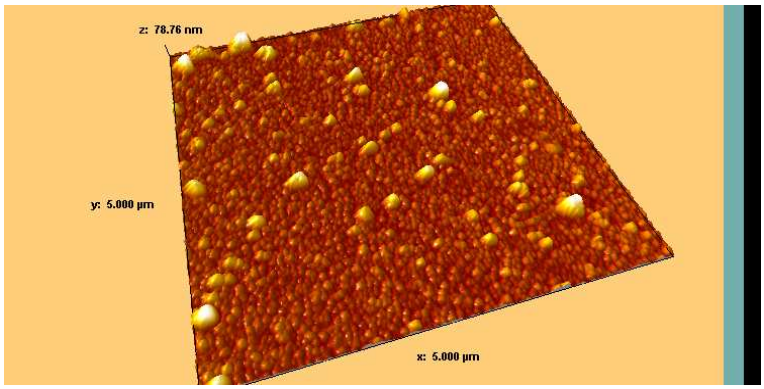
A higher displacement is seen in the pictures of the samples with L-cys (Fig. 4) and invertase (Fig. 5), $\sim$3 and 4 μm consequently. No arching effect has been seen in any of cantilevers. The deposited compounds are well and uniformly distributed on the golden surface.



a.



b.



c.

Fig. 2. AFM picture of the SiO_2 cantilevers: a – reference sample; b – sample with L-cys and c – sample with GSH.

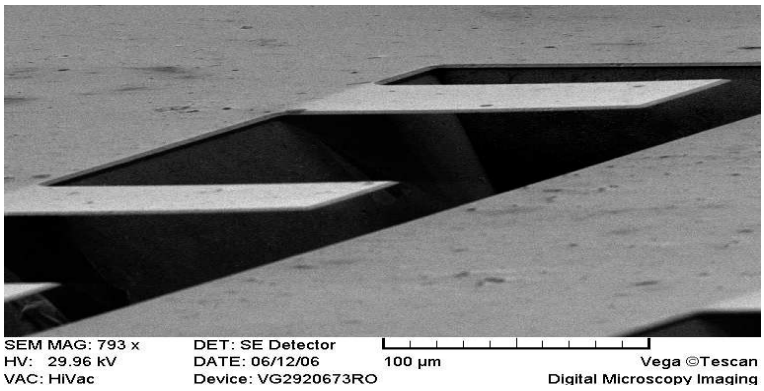


Fig. 3. SEM picture of the SiO_2 cantilevers (blank probe).

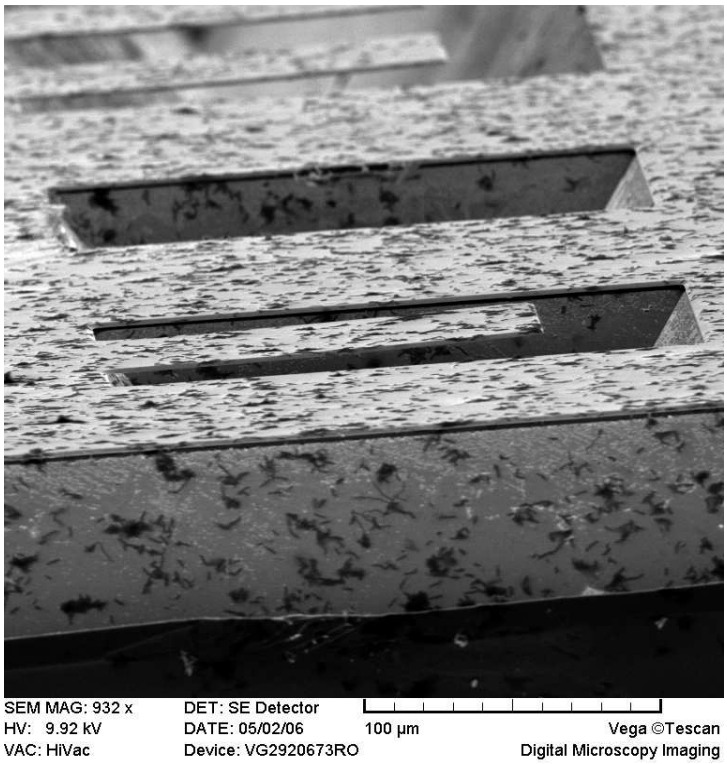


Fig. 4. SEM picture of probe with L-Cys.

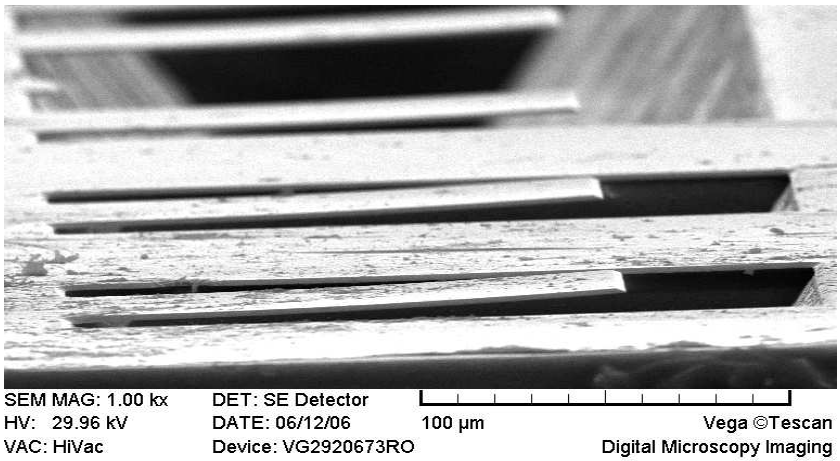


Fig. 5. SEM picture of Invertase covered cantilevers.

During one month storage of the cantilever array covered with GSH some micro-organisms have developed (Fig. 6). This prevents the proper measurement of the

deflection caused by GSH. Nevertheless, these results are relevant for this investigation, because of the observed backward deflection. It means that cantilever could be used not only to register by displacement resulting from binding of some substance to the surface, but also to distinguish between different compounds existing in the investigated flow.

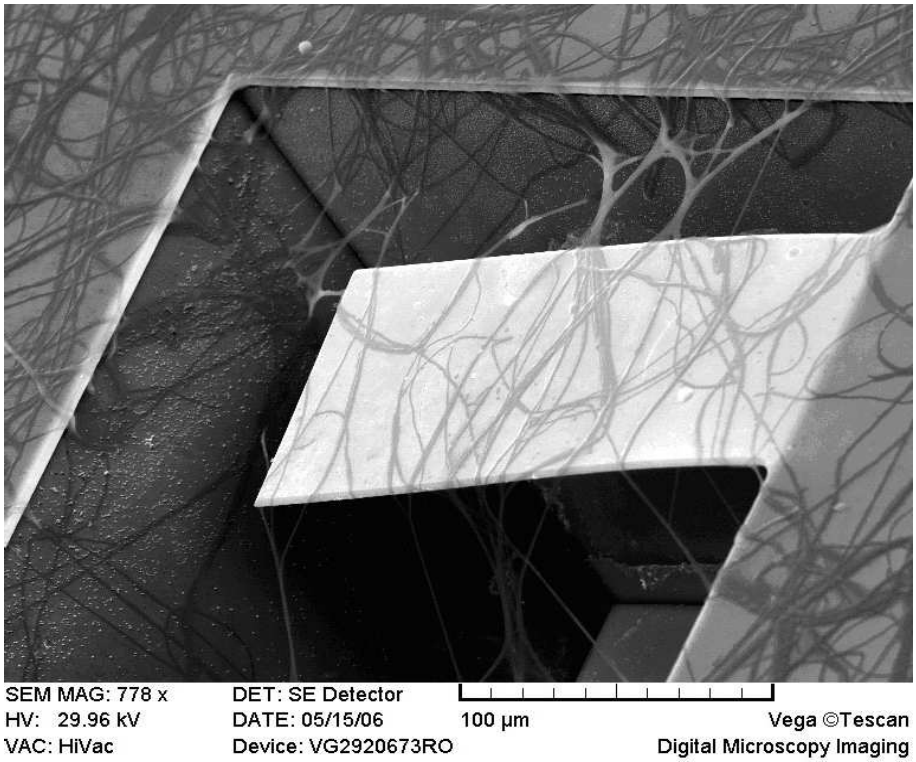


Fig. 6. SEM picture illustrating case of downward displacement of cantilevers with microorganisms.

3. Simulations

The displacements given by the binding of the biochemical compounds were measured using SEM pictures and the forces causing these displacements of the cantilever array were defined. Then the stresses of the structures were evaluated by simulations.

Simulations have been performed using the CoventorWare program with the following parameters: 1 000 volume elements; Type of elements: Parabolic Hexahedron 27V, 12PE, 6PF; Mesh type: Manhattan bricks Parabolic.

Because of the initial beam displacement of 2 micrometers one can conclude that a tensile stress in the cantilever exists. This displacement corresponds to a tensile stress of +20 MPa in Au (see Fig. 7).

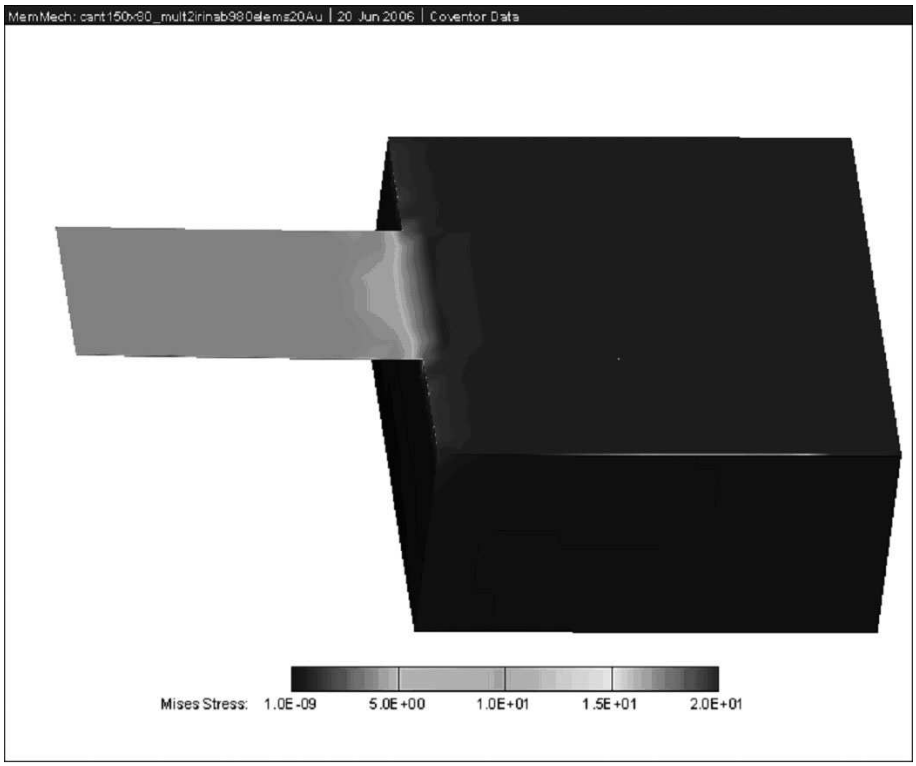


Fig. 7. Mises Stress of the cantilever in initial condition (displacement 2 μm given by the tensile stress only).

Simulations were performed taking into account the tensile stress for gold. Material properties used for the calculations are listed in Table 1.

Table 1. Material properties

Material	Density ρ [Kg/μm^3]	Young Modulus E [MPa]	Poisson ratio ν	Residual Stress σ [MPa]
SiO_2	2.2×10^{-15}	7×10^4	0.17	0
Au	19.3×10^{-15}	5.7×10^4	0.35	+20 (tensile)

A positive force of 1.6 μN was applied on the end of the bottom edge of the cantilever in order to obtain the 3 μm displacement for the cantilever covered with L-cys (Fig. 4 and Fig. 8) and a positive force of 2.4 μN applied on the end bottom edge of the cantilever for achieving 4 μm displacement in the probe with invertase (Figs. 5 and 9).

Taking into account the tensile stress for gold, a negative force of –8 μN applied on the end top edge was used to simulate the deflection shown in Fig. 6 (see Fig. 10).

The results for the stress caused by deposited layers on the Au and SiO_2 surface given by the simulations are listed in Table 2.

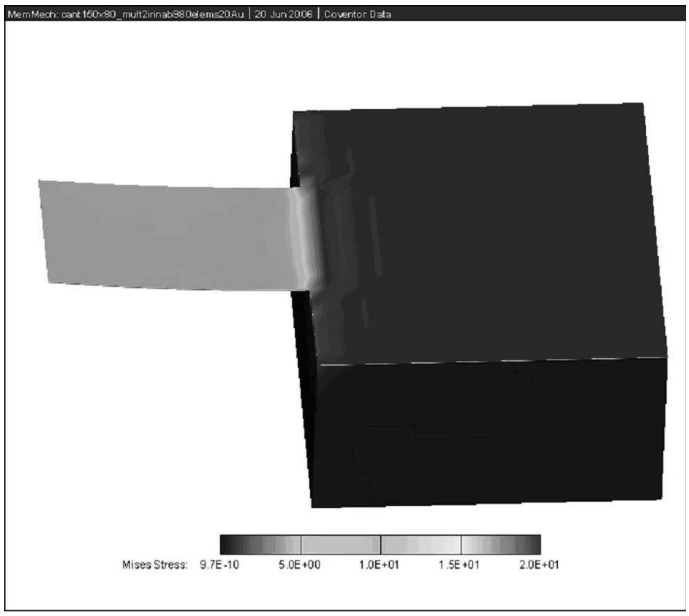


Fig. 8. Von Mises Stress of the sample with L-cys (3× deform displacement).

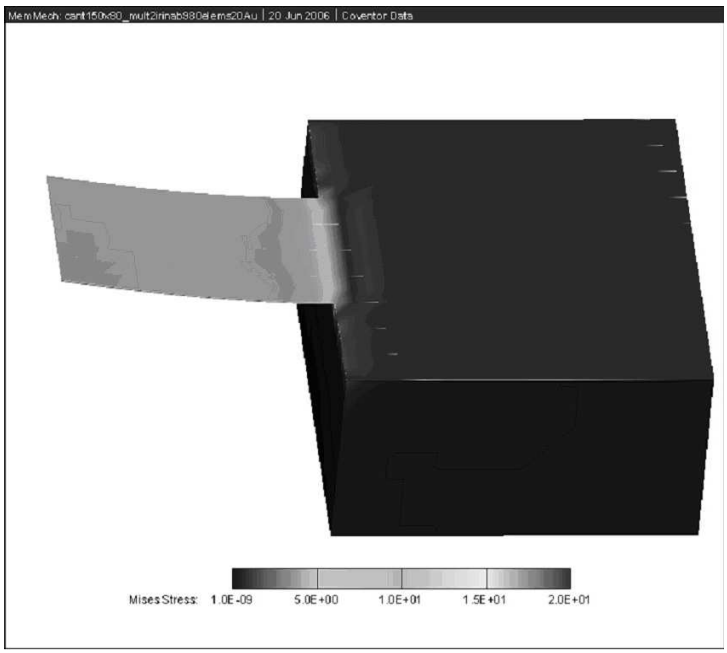


Fig. 9. Von Mises Stress of the cantilever with invertase (4× Deform Displacement).

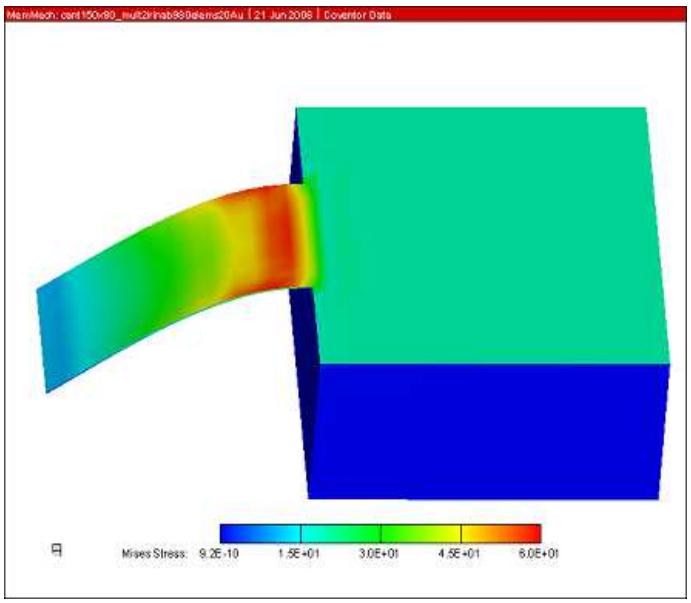


Fig. 10. Mises Stress of the cantilever with backward deflection (8× Deform Displacement).

Table 2. Input data of displacement and results of stress

	Displacement along Z axis [μm]	Applied force [μN]	Maximum values for von mises stress for the cantilever part only [MPa]	
			SiO_2	Au
150 μm length × 80 μm wide × 1.2 μm thickness	2 μm		12	18.5
	3 μm	1.6	14.6	17
	4 μm	2.4	16.8	17
	–9 μm	-8	60.4	59.4

Although in all the cases the applied force is less than the one used by other groups [4] the deflection is higher, proving the high sensitivity of the fabricated cantilever. Regardless of its small thickness, the predicted arching effect [4] was not observed. Keeping in mind that the fracture stress value for gold is 127 MPa (tensile) [8] and for SiO_2 is 9.52 GPa [9] one can observe that the simulated stress values are much smaller. This indicates that he cantilevers are reliable enough for detection purposes and will not break under normal working conditions required by the SAM formation and interaction with analytes.

4. Conclusions

A cantilever array with dimensions of the cantilevers 150×80×1.2 μm has been fabricated. Three different biochemical compounds were deposited and the cantilever

was investigated using SEM and AFM. Depending of the compound, a displacement in both directions between 2 and 9 μm has been observed. The arching effect was no observed. Simulations using CoventorWare have been done, and the computed stresses are in the range of 12 and 60.4 MPa. In conclusion, the results show that the cantilever is fairly sensitive and it can be used for biochemical application.

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