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Bio-functionalization of silicon nitride-based piezo-resistive microcantilevers

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Abstract. Methods of bio-functionalize silicon nitride involve process steps to convert it into an oxynitride via plasma implantation techniques. Such methods can potentially damage microstructures such as cantilevers. In this paper, we report successful bio-functionalization of Hotwire CVD silicon nitride-based piezo-resistive cantilevers without any oxygen plasma treatment. Process to fabricate such structures and to bio-functionalize them is discussed in detail.

Keywords. Hotwire CVD; microcantilever; antibody; piezo-resistive; immobilization; silanization.

1. Introduction

Microcantilever-based biosensors have attracted considerable interest to monitor a specific substance in applications such as clinical analysis, environmental control and industrial processes (Raiteri *et al* 2001). Adsorption of molecules onto the surface of the cantilever generates a small magnitude (5 mN/m to 0·5 N/m) surface stress, resulting in a bending of the cantilever (Fritz *et al* 2000). However, for the bending to occur it is necessary that the adsorption takes place preferentially on one surface of the cantilever (Raiteri *et al* 1999). The detection mechanisms could be optical (measure the deflection), or measurement of a change in resonant frequency of the cantilever due to mass change (Battiston *et al* 2001), or even piezo-resistive (change in resistance due to bending) (Thaysen *et al* 2001). However, when one applies the optical readout scheme to making measurements in liquids there are two important issues. Firstly, the laser system needs to be aligned afresh, whenever the refractive index of the liquid changes. Secondly, it is not possible to make a measurement in an opaque liquid as the laser would be absorbed by the liquid. For biomolecule detection by the change in mass technique, the expected change is very small (in femtograms); and to detect the consequent small change in frequency, one requires a cantilever with a high Q and a piezo oscillator stage with a large range (few MHz). Moreover, this detection technique does not detect at the point of care (PoC), and the instrumentation involved is quite sophisticated. Instead, if one

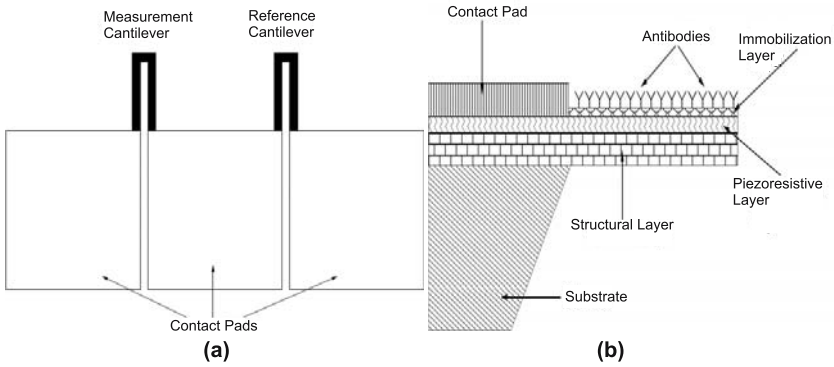

Figure 1. Schematic of the piezo-resistive cantilever **(a)** plan view, **(b)** cross-sectional view.

uses a piezo-resistive detection scheme one can circumvent the issues involved in the optical and mass change detection schemes. Alignment is not required; also issues of changing refractive index and processing in opaque liquids simply disappear. As the signal from the piezo-resistive detection scheme is an electrical signal; one can build appropriate electronics around it to amplify and process the signal.

In such devices Silicon nitride is routinely used as structural layers or encapsulation layers. It is therefore of interest to bio-functionalize silicon nitride, so that addition of an extra functionalization layer can be avoided. Techniques using oxygen plasma to form a thin layer of silicon-oxynitride on a Hotwire CVD deposited silicon nitride prior to silanization and antibody immobilization has been demonstrated by this group (Joshi *et al* 2004). Since it is desirable that such functionalization protocols do not pose any threat to the suspended structures in the device, the use of oxygen plasma might not be appropriate. In this paper, we report the fabrication of Hotwire CVD silicon nitride/polysilicon piezo-resistive microcantilevers and successful antibody immobilization on them without the use of oxygen plasma.

2. Experimental

The plan view in figure 1a shows two cantilevers connected in a half bridge configuration. Resistors are embedded in the two cantilevers. One cantilever can act as the measurement cantilever, while the other is the reference cantilever. The sectional view of a piezo-resistive cantilever is shown in figure 1b. The bottom layer acts as the *structural* layer, the *middle* layer is the embedded piezo-resistive layer and the top layer is a thin *immobilization* layer.

Materials required for fabricating the cantilevers were deposited by the Hotwire CVD technique. The material used for fabricating the structural layer was silicon nitride; the piezo-resistive layer was polysilicon doped *insitu* with boron. The films were deposited in a novel HWCVD cluster tool shown in figure 2. The polysilicon piezo-resistor was encapsulated using a top silicon nitride layer; further a chrome-gold layer was deposited to make electrical contact with the polysilicon layer. Process sequence was designed to ensure that the metal layers in the pad region made an electrical contact with the piezo-resistive layer. The top silicon nitride layer was then bio-functionalized so that antibodies could be attached on its surface. Antibody immobilization on the silicon nitride surface was verified by incubating fluorescence tagged antibodies on the cantilever.

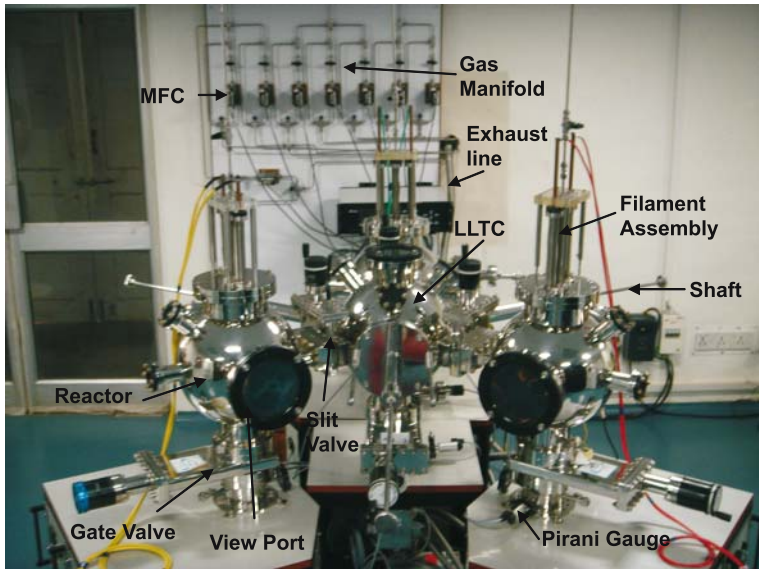

Figure 2. Hotwire CVD cluster system.

2.1 *Fabrication of the nitride/polysilicon/nitride piezo-resistive cantilever*

To fabricate the structure, a series of deposit, pattern and etch steps are employed. Figure 3a–j illustrate the main steps of the fabrication process. First, a 2 inch diameter ⟨100⟩ oriented, silicon wafer was cleaned with piranha (1:3 H_2O_2 : H_2SO_4). Next, a stack of silicon nitride/doped polysilicon/silicon nitride thin films was deposited on the silicon substrate (figure 3b). The films were deposited at process parameters given in table 1.

The cantilever pattern and the contact pad region were defined by photolithography. Then, the nitride/poly/nitride stack was patterned by reactive ion etching (RIE); (figure 3c). The etch chemistry for silicon nitride was $CF_4 + CHF_3$, whereas polysilicon was etched using $CF_4 + O_2$. The photoresist was stripped by sonication in acetone; in some cases the photoresist residues were required to be removed by oxygen plasma ashing.

It is necessary to open contact windows in the pad region so that an electrical connection can be established with the embedded piezo-resistor. This was achieved by defining a pattern, using photolithography, to etch the nitride in the pad region. Nitride in the pad region was again etched using $CF_4 + CHF_3$ chemistry in RIE (figure 3d). This was followed by the photoresist strip step. Chrome–gold was then sputtered on the wafer using the Nordiko sputter tool. The thickness of this layer was about 100·nm. The contact pads were defined by the third level mask of photolithography. Gold was etched using a solution of potassium iodide (KI) and iodine in water; while the chromium layer was etched using a solution of ceric ammonium nitrate (CEN) and acetic acid in water.

2.1a *Bulk micromachining:* The cantilevers were released by the process of bulk micromachining (figure 3g). However, before this step the photoresist was stripped and any native oxide was etched by dipping the wafer in 2% Hydrofluoric acid. The wafer was then cleaned with DI water and then immersed in a reflux container for bulk micromachining. The etchant used was a 10% solution of TMAH (Tetramethyl ammonium hydroxide). The temperature of

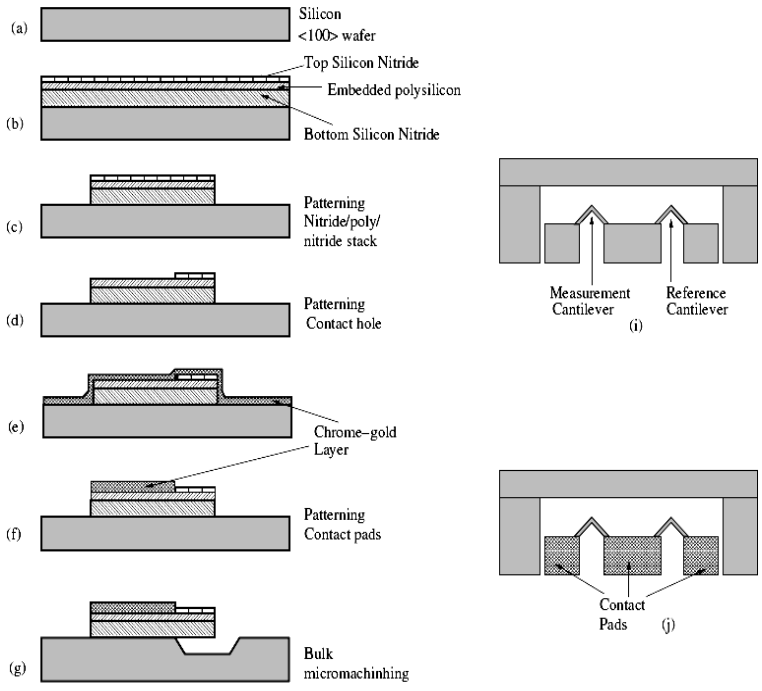

Figure 3. Process flow highlighting the main steps in fabricating the piezo-resistive cantilevers.

the water-bath was maintained at 86°C and the etch step was carried out for 25 minutes. The wafer was then transferred into water.

2.1b *Release:* As the water evaporates, the free structures can collapse due to stiction. This is due to capillary forces and high surface tension of water. Therefore, the wafer was

Table 1. Process parameters to deposit stack of HWCVD thin films.

	Bottom silicon nitride structural layer	Embedded piezo-resistive layer	Top silicon nitride immobilization layer
Base pressure	8×10^{-7}mbar	8×10^{-7}mbar	4×10^{-7}mbar
Silane: Ammonia flow rate ratio	1:20	NA	1:20
Silane: Hydrogen: Diborane (10% in H_2) flow rate ratio	NA	5:100:2	NA
Process pressure	6×10^{-2}mbar	$1{\cdot}1 \times 10^{-1}$mbar	6×10^{-2}mbar
Substrate heater temperature	300°C	300°C	300°C
Filament temperature	1850°C	1850°C	1850°C
Time	30 minutes	20 minutes	3 minutes
Expected thickness	180 nm	120 nm	18 nm

transferred into a low surface tension liquid (methanol/isopropyl alcohol) and then this liquid was allowed to evaporate. In a few release runs the alcohols were heated lightly, to about 60°C, to further reduce their surface tension. The structures were released *only* when the alcohols were fully evaporated.

2.2 *Bio-functionalization of silicon nitride*

The process to biofunctionalize the silicon nitride surface is described as follows.

2.2a *Sulphochromic acid treatment:* The cantilever was subjected to treatment by sulphochromic acid for 10 minutes, which presumably creates silanol sites on silicon nitride surfaces. The samples were dried by heating under vacuum.

2.2b *Silanization:* The dehydrated samples were dipped in 1% silane (N-[3-(Trimethoxysily) Propyl-Ethelene Diamine, 97%) solution for five minutes. The silanized cantilevers were rinsed in ethanol in order to remove excess silane on the surface, and dried. Condensation was carried out by heating the samples at 110°C for 10 minutes in argon ambient.

2.2c *Linker attachment:* Dip the cantilever die in a cross linker (1% aqueous solution homobifunctional agent glutaraldehyde) for 30 minutes. Thus linker molecules get attached to the surface amine sites, leading to free aldehydes on the surface for biomolecules to bind.

2.2d *Antibody immobilization:* Human immunoglobulin (HigG) antigens were then immobilized on the modified cantilever surface by incubating them on the cantilever surface for 1 h. Loosely adsorbed biomolecules on the surfaces were washed with a detergent solution (0·1% aqueous solution of Tween-20) and rinsed with PBS.

2.2e *FITC antibody attachment:* To identify the grafted antibody layer, FITC tagged goat anti-HIgG (1 ml/ml in PBS) was allowed to react with the sample surfaces for 1 h. The die was again rinsed. If the FITC tagged antibodies have attached to the corresponding antigens, they are visible under a fluorescence microscope.

3. Results and discussion

The released silicon nitride/polysilicon/silicon nitride piezo-resistive cantilever structure is shown in figure 4. It should be noted that the entire cantilever stack was fabricated using the thin film materials deposited in the Hotwire CVD cluster system that was shown in figure 2. In figure 5 we shown the SEM image of the die that consists of the basic piezo-resistive sensor device, along with the electrical contact pads. The triangular structures are microcantilevers that are connected in a half Wheatstone bridge configuration. The cantilever stack is made up of: (i) bottom 180 nm thick silicon nitride structural layer; (ii) embedded 80 nm piezo-resistive polysilicon layer; and (iii) top 20 nm silicon nitride passivation layer. Also shown in the image are the gold pads that make an electrical contact with the embedded piezo-resistive layer. Figure 6 depicts a nitride/polysilicon/nitride cantilever as observed under a fluorescence microscope. The surface of this cantilever was modified by the silanization protocol, HIgG antibodies were immobilized on it, and FITC tagged antiHIgG were allowed to incubate on it. We observe dense and uniform fluorescence in the cantilever region; however, fluorescence is not observed in the pad region and the pit. This fluorescence image is an evidence of the

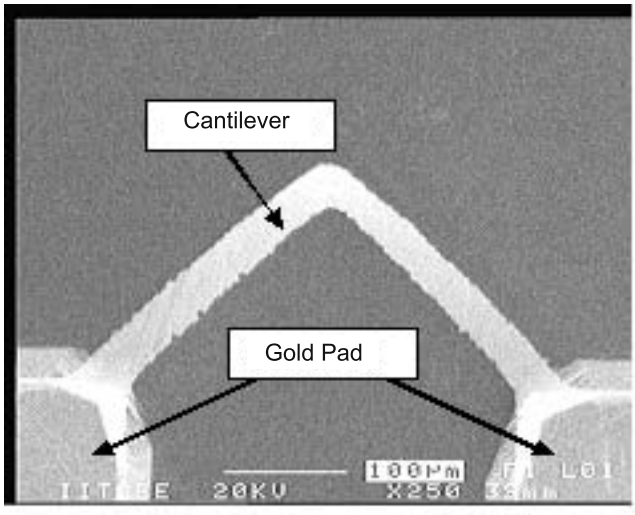

Figure 4. A released nitride/polysilicon/nitride cantilever.

fact that FITC tagged antibodies were successfully attached to the HIgG antigens that were immobilized on the surface of the nitride/poly/nitride cantilever.

4. Conclusion

Piezo-resistive microcantilevers comprising a structural silicon nitride layer, a piezo-resistive polysilicon layer, and an encapsulating silicon nitride layer were successfully realized and fabricated. All the three layers were deposited by the Hotwire CVD method. The encapsulation silicon nitride layer was bio-functionalized via the process of silanization. Human immunoglobulin HIgG antibgens were successfully immobilized, as evidenced by the fluorescence micrographs, on the encapsulation layer of the cantilever. Most importantly,

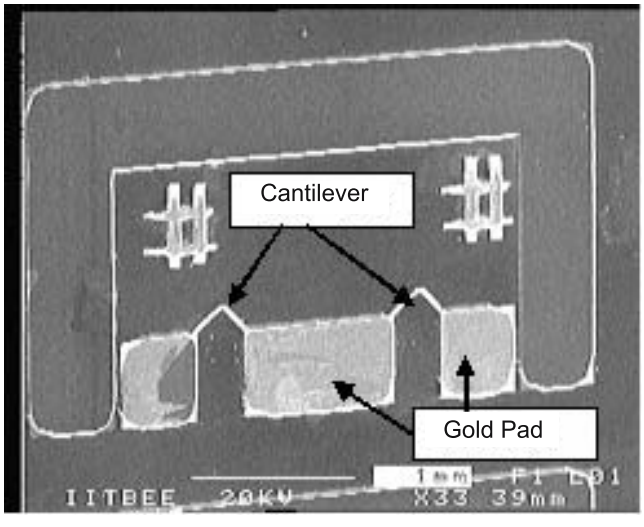

Figure 5. Full die showing the piezo-resistive cantilevers connected in half Wheatstone bridge configuration.

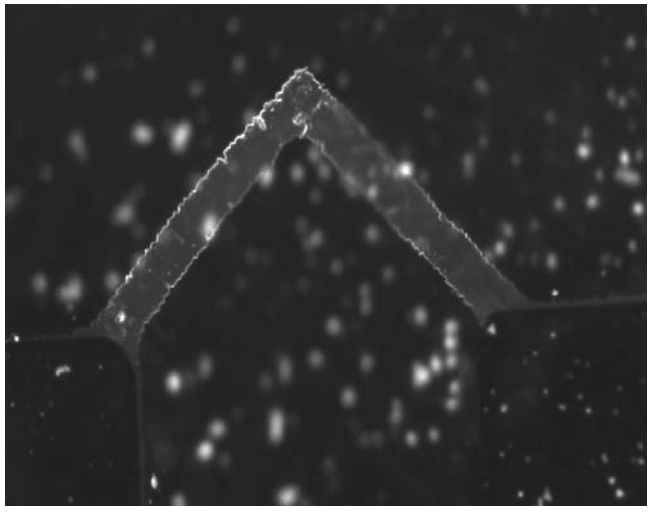

Figure 6. Fluorescence microscope images of FITC tagged anti-HIgG antibodies immobilized on a nitride/poly/nitride cantilever bio-functionalized by the process of silanization.

antibodies were successfully immobilized on the nitride surface without taking recourse to oxygen plasma treatment, thereby protecting the suspended microstructures from a potentially damaging process.

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