Assessment of Protocols for antibody immobilization on Au surface in SPR sensor: Although the results of sucrose experiments have been consistently good in our setup, the antigen-antibody experiments did not produce consistent and reproducible results. In order to find out the reason behind this, characterization of the surface was performed at various stages of the preparation process.

Sensor surface preparation
The BK7 glass coverslips are thoroughly cleaned by sonication in acetone followed by DI water rinsing and Nitrogen jet drying. The cleaned glass coverslips are then sputtered with a bi-metal layer of Ag and Au in the metal sputtering system (NORDIKO). The coverslips are initially sputtered with Ag for 20 seconds at 100 W followed by Au for 20 seconds at 80W. The sputtered metal bi-layer is 45 nm thick with 15 nm thickness of Ag and 30 nm of Au.

The AFM (Nanoscope IV) image of the coverslip is shown below. The surface roughness observed is 1.60946nm.

Sucrose calibration experiments on these surfaces showed sensitivity in the range of 10 micro-RIU (refractive index units).
**Antibody immobilization on the sensor surface**

The Cysteamine - Glutaraldehyde protocol which was adopted earlier was assessed for suitability in use with our SPR system. In order to confirm the presence of immobilized antibodies on the surfaces (immobilized using this protocol), the coverslips were incubated with FITC tagged HlgG (50 μg/ml in PBS) for 20 min. Fluorescence images were observed using the ZEISS Axioskope-2 MAT. Fluorescence images were acquired by using a Peltier-cooled black and white digital camera. As it can be seen from the figure below, antibody immobilization and antigen interaction was very scattered and non-uniform. This can possibly give rise to non-reproducible results in SPR.

![Fluorescence image of GaHlgG – HlgG(FITC) Binding](image)

Accordingly, a dendrimer based approach for immobilization is being tried out.

**G4 Dendrimer based approach:**

Sensor Surface Preparation: The coverslip sputtered with Ag-Au layer as explained earlier. This coverslip is first dipped in acetone for 15 minutes, then in ethanol for 15 minutes followed by DI water washing and Nitrogen jet drying.

**Protocol for anchoring PAMAM(G4) on gold surface:**

1. A self assembled monolayer of Thiols (SH group) is formed on the gold surface by immersing the cleaned coverslip in 20mM Cysteamine (HSCH2CH2NH2) in PBS for 2 hours. This is followed by PBS wash.
2. The coverslip is then immersed in 10 % Glutaraldehyde solution prepared in DI water for 1 hour. This is followed by DI water rinsing.

3. On this coverslip 100 μl of G4 dendrimer in methanol is poured and incubated for 1 hour.

The AFM image obtained is as follows. The surface roughness observed is 2.37313 nm.

![AFM image of Ag-Au coverslip with dendrimer](image)

**Protocol for functionalization of PAMAM:**

1. The PAMAM anchored coverslip is then immersed in 10 % Glutaraldehyde solution prepared in DI water for 1 hour. This is followed by DI water rinsing.

2. These coverslips are incubated in 100 μg/ml Goat anti Human Immunoglobulin–G (GaHIg-G) solution prepared in PBS (pH 7.4) for 12 hours.

3. After 12 hours, the coverslip is washed with PBS.

In order to confirm the presence of immobilized antibodies on the surfaces, the coverslips were incubated with FITC tagged HIgG (50 μg/ml in PBS) for 20 min. Fluorescence images were observed using the ZEISS Axioskope-2 MAT. Fluorescence images were acquired by using a Peltier-cooled black and white digital camera. In this case also the interaction is somewhat non-uniform, although much greater in density that the earlier protocol. It will be our aim to adapt this protocol to elicit an uniform interaction over the surface.
FABRICATION AND CHARACTERIZATION OF NOVEL POLYMER COMPOSITE MICROCANTILEVER SENSORS FOR EXPLOSIVE & iSens DETECTION

In the last 6 months, we further worked on the development of a SU-8 based novel polymer composite microcantilever sensor designed for surface stress measurements. Nanoindentation study was carried out for measuring the Young’s modulus of the polymer composite. A low cost process, optimized for fabrication of composite SU8 microcantilevers with thickness as small as 3 µm is developed and characterized as part of this work. Further, we also demonstrate the application of this polymer composite cantilever for explosive and cardiac marker detection with the appropriate surface coatings carried out on the polymer surface.

INTRODUCTION

Microcantilevers are simple MEMS devices with potential applications for development of many physical, chemical and biological sensors [1, 2]. The sensing principle for microcantilever sensor is based on the translation of analyte-target interaction into a nanomechanical motion that can be measured by optical or electrical transduction schemes. Microcantilever sensors with an optical transduction scheme are expected to offer the highest sensitivity. However, because of the practical limitations of optical transduction schemes such as difficulty in integrating the cantilevers into a miniaturized biochemical analysis system, electrical transduction methods using piezoresistive materials are normally preferred for sensing the cantilever deflection. Microcantilever with an integrated piezoresistor performs electrical transduction of strain by a resultant change in resistance. Therefore, when a microcantilever with its surface functionalized with a coating that is selective to the target molecules is exposed to the analyte, molecular adsorption results in a differential surface stress between the top and bottom surfaces of the microcantilevers. This differential surface stress results in a change in resistance of the piezoresistive layer. Surface stress sensitivity increases for the microcantilevers with increase in the gauge factor (K) of the piezoresistive films and decreases with the Young’s modulus (E) of the structural materials. Though silicon based materials are
normally used for fabrication of micro-cantilevers, polymers such as SU-8 have a much lower E (approx. 40 times lower compared to silicon) and hence are preferred because of their higher sensitivity [3].

SU-8 microcantilever with an integrated gold strain gauge is one of the electrical transduction approaches used in polymer microcantilever technology [3]. In order to address the problem of lower sensitivity attributed to the lower gauge factor of gold, polymer microcantilevers with an integrated low temperature polysilicon piezoresistor was introduced recently [4,5]. However, since polysilicon is a stiffer material, the thickness of the piezoresistive film needs to be kept low in order to not increase the stiffness of the structure. However, thinner polysilicon would add to the noise during measurements and hence reduces the signal to noise ratio. In this scenario, there is a scope for introducing a strain sensitive material with lower young’s modulus and higher gauge factor. Gammelgaard et.al reports the use of a composite film of Carbon black (CB) in SU-8 as a strain sensitive layer in SU-8 cantilevers with a total thickness of 7 μm [6]. Better sensitivity had been shown by improving the dispersion of CB in SU-8 and reducing the thickness of the cantilever structure, which was the focus of our previously published paper [7]. In this work we present a novel process flow which reduces the process complexity and allows realization of sensitive and cost effective sensors by an improved device design and fabrication techniques. Like in the case of structural material SU-8, the mechanical characterization of the strain sensitive SU-8/CB composite film is essential in order to understand and estimate the mechanical behavior of this MEMS device. Electrical and mechanical characterization of polymer composite film aids in optimizing the CB concentration in SU-8 for the strain sensitive layer.

This paper reports the fabrication and characterization of piezo-resistive SU-8 microcantilevers optimized for surface stress measurements using an SU-8/Carbon Black composite as the strain transduction layer. Mechanical characterization for estimating the Young’s modulus of this polymer composite using nanoindentation is reported for the first time. This paper also demonstrates the application of this polymer composite cantilever for explosive detection with the appropriate surface coatings carried out on the polymer surface.

**SU-8 /CARBON BLACK COMPOSITE**

Polymer composite is prepared by ultrasonic mixing of Carbon Black (CB) Conductex 7067 Ultra(Columbian Chemicals) in SU-8 and Microchem Nanothinner. The mixing parameters are optimized to get a uniformly dispersed spin coatable polymer composite. Like SU-8, this material can be photolithographically patterned. Scanning electron micrograph of the composite with CB concentration of 8 vol% is shown in Fig.1.
The basic mechanical characterization of these thin film composites was performed by nanoindentation with a Hysitron Triboscope. The aim of the experiment was to check whether CB filler loading would potentially change the Young’s modulus of SU-8 composite. SU-8/CB composite samples with different CB concentrations were prepared and each sample was subjected to a set of indents using Berkovich indenter yielding a load-depth curve (Fig. 2). Olivar Pharr method was used to analyze the unloading part of the load-penetration depth curve in order to extract the reduced modulus, Er. Young’s modulus of the composite thin film, Ef is calculated from Er by taking the substrate effect into consideration using a modified King’s analysis [8] using (1).

\[
\frac{1}{E_r} = \frac{1-v_s^2}{E_s} + \frac{1-v_i^2}{E_i} \left( 1 - e^{\frac{a(t-h)}{a}} \right) + \frac{1-v_s^2}{E_s} \left( e^{\frac{a(t-h)}{a}} \right) \]

(1)

Es and Ei are the moduli of the substrate and indenter respectively. Parameters a, t and h are square root of projected area, film thickness and indenter depth respectively.

The modulus values are compared with existing theories for particulate filled polymer
composites [9, 10].

Figure 3 (a) Young’s Modulus of SU-8/CB composite from nanoindentation as a function of CB vol %. (b) Berkovich indent at the SU-8 surface.

Modulus increased by 35% for the 8 vol% sample. Electrical characterization experiments were carried out to understand the conductivity behavior of these composites at different CB loading and the percolation threshold was calculated to be 6 vol%. Average resistivity for 8 vol% sample was 0.7 Ω-m.

MICROCANTILEVER FABRICATION

The design of the cantilever is chosen in order to improve the surface stress sensitivity, common mode rejection, mechanical stability and packaging compatibility of the sensor. Fabrication of these devices involves five levels of lithography (fig. 4).

Fabrication process begins with piranha cleaning of a 2” silicon wafer which is used as a dummy reusable substrate for the polymer structure to be fabricated. The wafer is subjected to thermal oxidation or dielectric sputtering of silicon dioxide and this layer
becomes the sacrificial layer that would be finally etched away in order to release the cantilever die from the substrate. SU-8 2000.5 was spin coated and baked at 650C and 850C for optimized timings using a slow ramp-up and ramp-down to room temperature to reduce the thermal stress. Then the samples were subjected to UV exposure using the corresponding mask for layer 1 of the SU-8. The sample was subjected to a post exposure bake cycle, development and a final iso propyl alcohol (IPA) rinse step to yield the first structural layer of microcantilever. This is followed by the patterning of gold contacts for the piezoresistor. Strain sensitive polymer composite layer is prepared by spin coating and patterning of SU-8/CB composite with CB concentration of around 9 vol%. This is followed by an additional ultrasonic cleaning step in IPA in order to remove the CB residues. This layer is encapsulated by another structural layer of SU-8 followed by a final SU-8 2100 patterning which forms the supporting anchor. Microcantilever die is obtained after a final etch step of the sacrificial silicon dioxide in a buffered hydrofluoric acid to release the polymer structure from the substrate. The SU-8 frames containing arrays of SU-8 microcantilever chips were rinsed in DI water and isopropyl alcohol and allowed to dry. The fabricated microcantilevers are just 3 µm thick. Optical and SEM images of the fabricated devices are shown in fig. 5.

![Figure 5 Images of fabricated devices (a) Arrays of SU-8 device chips (b) Optical (c) SEM image of one of the device chips containing 4 cantilevers](image)

**CHARACTERIZATION**

The fabricated microcantilevers were electromechanically characterized to demonstrate the piezoresistive behavior. Deflection sensitivity which is an important performance parameter for piezoresistive microcantilevers is a measure of the relative change in resistance ($\Delta R/R$) of the embedded piezoresistor as a function of cantilever deflection. The measurement was performed by deflecting the cantilever using a calibrated micromanipulator with a resolution of 10 µm with simultaneous measurement of resistance. The measurements were performed using HP 4200 parametric analyzer while probing the microcantilevers inside a low noise shielded probe station from Suss Microtech. Since the resistance of polymer composite material exhibited bias voltage dependency, $\Delta R/R$ for three different bias voltages were plotted as a function of deflection.
Deflection sensitivity extracted from these measurements (fig. 6) is more than three times the sensitivity of SU-8 cantilevers with gold as strain gauge [3].

In order to use these devices for detecting explosive vapors such as tri nitro toluene (TNT), microcantilevers need to be functionalized with some receptor molecules. 4-mercaptopentanoic acid (4-MBA), which can form self assembled monolayer on gold, is one of the receptor molecules reported in literature [11]. For this purpose, microcantilevers were coated with 25 nm of gold with 5 nm of titanium as adhesion promoting layer. These gold coated microcantilevers were then functionalized with 4-MBA and stored inside a humidity controlled environment.

The devices are then connected in a DC bridge circuit and incubated inside a gas flow cell for explosive vapor experiment. Dry Nitrogen purging was done for an hour before starting the experiment in order to bring down the humidity levels. The experiments were conducted at a carrier nitrogen gas flow of 30 SCCM, controlled using a mass flow controller and the TNT concentration levels were approximately a few ppb. Microcantilever response recorded during consecutive cycles of exposure to TNT and nitrogen is given in fig. 7. The sensor was getting regenerated in post nitrogen purging step.
Summary:

Polymer composite microcantilevers aiming for surface stress measurements were fabricated with an average composite beam thickness of just 3 µm. Mechanical characterization of polymer composite was performed using nanoindentation technique and this helped in estimating the Young’s modulus of the composite for different CB concentrations. Electromechanical characterization of a sample microcantilever was performed to demonstrate the piezoresistive transduction of the fabricated device and the computed deflection sensitivity from these measurements was approximately 1 ppm/nm. Microcantilever application for explosive vapor detection was also demonstrated by functionalizing the microcantilever followed by exposure to TNT vapor with simultaneous recording of microcantilever piezoresistor output.

REFERENCES

**iSens: A Point of Care sensor for detecting Cardiac Markers**

**Report of the activities for the period Sept 2009 – Feb 2010:**

The project consists of 5 major components: (a) Fabricating Piezoresistive Microcantilever devices; (b) Achieving Antibody Immobilization on Microcantilever Surface; (c) Fabricating liquid cells with inlets and outlets, to house the cantilever and to perform Bio-experiments; (d) Building electronics to detect $\Delta R/R$ of the order of 5ppm; and (e) Integration of the above components.

Piezoresistive microcantilevers were successfully realized using SU-8 + Carbon Black. Antibodies (HIgG and Myoglobin were immobilized on the SU-8 cantilever surface via the silanization protocol. Liquid cells were fabricated using PDMS with inlets and outlets with the capability that the cell was properly sealed. The SU-8 + Carbon Black cantilevers were integrated in this liquid cell and bio-experiments were performed i.e. antigens were introduced into the composite device (Liquid cell + cantilever). This setup was integrated with the $\Delta R/R$ measurement setup (currently it can measure 5ppm) and experiments for detecting were conducted.

The SU-8/Carbon black/ SU-8 cantilevers (bare, and immobilized with antibodies are shown below):

![Before Antibody Immobilization](image1.png)  ![After Antibody Immobilization](image2.png)

The PDMS Liquid Cell fabricated using a Poly-di-methyl-siloxane (PDMS) elastomer is shown below: The fabrication process is simple (optical Lithography, Plasma treatment, etc.); the cell volume is about 10-20 $\mu$L. The main advantages of using PDMS are: it is Bio-compatible and patternable, its surface-modification is simple; and it is transparent (so some microscopy can be done).
The integrated system with its associated electronics was set up to conduct the bio-experiments. The set up is shown below:

[Images of microsyringe pump, cantilever sensor in fluid cell + electronics, and computer interface]
The results of bio-experiments are shown below: (1). Concentration parameters are: HlgG = 0.1mg/ml and FITC = 0.05 mg/ml. And (2). Anti myoglobin = 0.5mg/ml and Myoglobin = 0.5 mg/ml

Thus we obtain some preliminary indication that the biosensor is working. Additional experiments will be performed to verify the results.