# CHITOSAN FOR MEMS
## *Demonstration of Micromechanical and Optical Biosensors*

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Abstract: This paper presents the biological functionalization of MEMS sensors by using the polysaccharide chitosan. Chitosan is a unique polymer due to its abundance of primary amine groups and its ability to be electrodeposited with spatial and temporal control. Biomolecules such as DNA and proteins can be attached to chitosan films by standard coupling chemistries. This biofunctionalization approach was demonstrated for two different MEMS devices: a microcantilever and an optical sensor. The devices were coated with chitosan and probe DNA and were used for detecting the hybridization with target DNA. Here, we describe the design, fabrication procedure, and testing results for both of these biosensors.

## 1 INTRODUCTION

Functionalizing surfaces for the detection of specific analytes is a common practice for many biosensor devices. Electrochemical sensors, for example, often employ hydrogels which selectively block the diffusion of particular molecules or entrap enzymes to enable specific detection (Geng et al., 2008). It is a greater challenge to functionalize surfaces of microelectromechanical systems (MEMS) sensors due to their small size. MEMS sensors hold many advantages over their macroscale counterparts, including low cost due to batch fabrication techniques, high-throughput screening ability, and small required sample volumes. Several functionalization schemes have been demonstrated for MEMS devices (Mizutani, 2007), including the self-assembly of thiol labeled molecules to gold surfaces and of silane labeled molecules to silica surfaces. These techniques require time consuming laboratory procedures to ensure the integrity of the biomolecules and offer limited control over their patterning.

We report the use of an amine rich polysaccharide, chitosan, to functionalize surfaces in both mechanical and optical MEMS biosensors. Chitosan can be selectively electrodeposited on patterned conductive surfaces, and it has primary amine groups at every repeating sugar unit of its polymer structure (Yi et al., 2005). The amine groups can be used for covalent attachment of various biomolecules, making chitosan an excellent interface between microfabricated devices and biological components. Here, we present the attachment of probe DNA to chitosan-coated sensors used for the detection of target DNA.



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## 2 CHITOSAN PROPERTIES

Chitosan is derived from the partial deacetylation of chitin, an abundant material found in nature. At low pH below a pKa value of 6.3, chitosan is cationic and soluble in water. However, as the pH rises, chitosan becomes protonated and insoluble. We take advantage of chitosan's pH dependent solubility to electrodeposit a chitosan film with spatial and temporal control in the MEMS sensors. In an electrochemical reaction, the pH at the cathode surface will rise due to the reduction of the hydrogen ions. This rise in pH will cause a film of chitosan to form over the conductive surface with a rate dependent on the applied bias (Yi et al., 2005).

## 3 MICROMECHANICAL SENSOR

### 3.1 Design

The cantilever sensor consists of layers of $Si_3N_4$ (500nm thick), Au/Cr (100nm thick), and chitosan with probe DNA (~100nm) on a silicon substrate as shown in Fig. 1 (Koev et al., 2007). The cantilever length and width are 100µm and 40µm respectively. When exposed to target DNA with a complementary sequence to the probe, the target binds to the probe and causes two different effects that can be used for detection. First, the mass of the cantilever is increased, causing a drop in its resonant frequency (dynamic mode detection). Second, the surface stress is increased, causing the cantilever to deflect (static mode detection). The cantilever displacement and resonant frequency are measured with an optical interferometer (Veeco NT1100). For resonant frequency measurement, the device is electrostatically actuated by applying a voltage between the gold layer and the substrate.

### 3.2 Fabrication

The cantilever is fabricated on a 4 inch Si wafer with two contact lithography steps. First, a layer of $Si_3N_4$ is deposited by chemical vapor deposition (CVD), and layers of Cr and Au are deposited by sputtering. The metal is patterned by wet chemical etching, and the $Si_3N_4$ is patterned by reactive ion etching. The cantilever is released by etching the Si substrate with KOH. Chitosan is deposited on the fabricated device by immersing it in an acidic chitosan solution and applying a negative voltage. Amine-labeled probe DNA is attached to the chitosan with glutaraldehyde as a crosslinker.

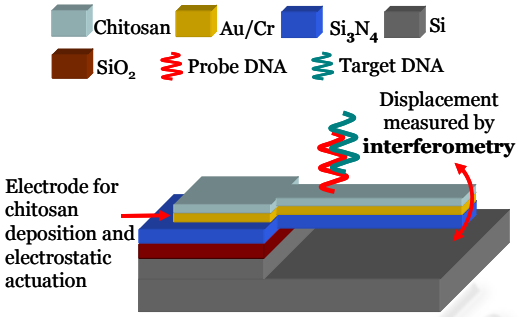


Figure 1: Cross sectional schematic of microcantilever sensor.

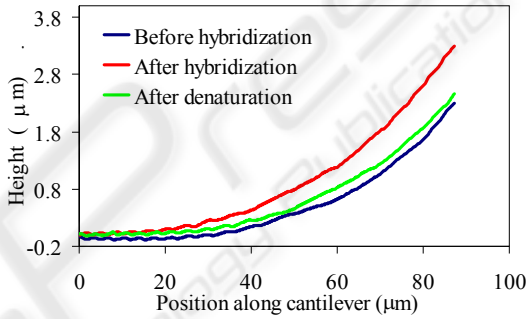


Figure 2: Static response of cantilever to complementary DNA hybridization and denaturation.

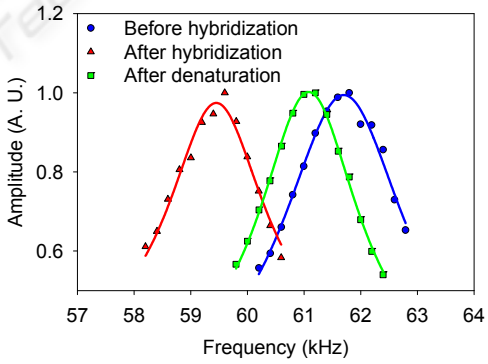


Figure 3: Dynamic response of cantilever to DNA hybridization and denaturation.

### 3.3 Testing and Results

The device is immersed in a complementary target DNA solution for hybridization and in a urea solution for denaturation (Koev et al., 2007). After each step, the device is rinsed with deionized water and dried. Then, the resonant frequency and the bending profile of the dried cantilever are measured by interferometry as described previously. Fig. 2 shows the static response to DNA hybridization, and

Fig. 3 shows the dynamic response. Both responses demonstrate that biological recognition occurred and was transduced to a mechanical signal.

# 4 OPTICAL BIOSENSOR

## 4.1 Design

Our design for the biophotonics platform is shown in Fig. 4 (Powers et al., 2005). A thick photosensitive polymer, SU-8, is used to define both the fluidic channel and the optical waveguide on a pyrex substrate. SU-8 is ideal for this application due to its chemical inertness, ability to be spun very thick, and high optical transmission for visible wavelengths. Pyrex is chosen due to its smaller index of refraction compared to the SU-8 (1.47 to 1.59). As shown in the schematic, excitation light is applied through the same optical fiber which collects the fluorescence emission through the use of an off-chip bi-directional coupler. In order to maximize the collection efficiency at the waveguide facet, the chitosan is deposited onto the sidewall of the SU-8 channel. A film of indium tin oxide (ITO) is chosen to facilitate this deposition. Indium tin oxide is conductive and mostly transparent to visible wavelengths. The height of the SU-8 is chosen to be 110 µm in order to adequately couple the emitted fluorescence into a multimode fiber with a core diameter of 62.5 µm.

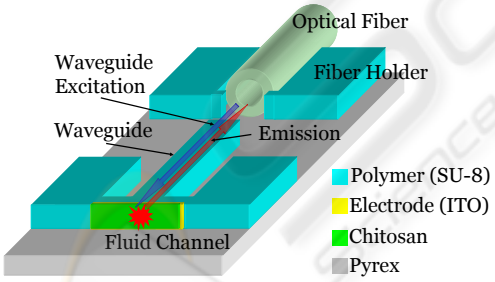


Figure 4: Schematic of the optical biosensor.

## 4.2 Fabrication

Gold electrodes are initially patterned on a 4 inch pyrex wafer using standard lithography techniques. Prior to the addition of the SU-8, an adhesion promoter AP300 (Silicon Resources, USA) is spun on the wafer and baked on a hotplate in order to improve the adhesion between the SU-8 and the pyrex. SU-8 is spun to a thickness of 110 µm and patterned to create the waveguides and fluidic channel. Indium tin oxide is deposited using magnetron RF sputtering to achieve a final thickness of 200 nm. The sidewall of ITO is created by patterning AZ9245 photoresist over the features on the wafer and etching away the exposed ITO in a 1:1 HCl:DI water solution. In order to functionalize the ITO surface, the wafer is submerged in a chitosan solution at a pH of 5.07 (Sigma-Aldrich, USA) while a voltage bias of 2 VDC is applied to the electrodes. After 15 minutes, a film of chitosan on the order of a few microns thick is deposited onto the ITO electrode. The wafer is rinsed with DI water to remove any excess residues. An optical fiber is aligned to the output waveguide by a patterned fiber clamp structure and is glued in place using UV-curable epoxy. The index-matching epoxy fills the gap between the fiber and the waveguide to eliminate losses due to reflections.

## 4.3 Testing and Results

The optical biosensor was tested in response to attachment of probe DNA and to hybridization with target DNA (Badilita et al., 2007). Amine labeled fluorescent probe DNA is attached to the chitosan by glutaraldehyde crosslinking. The optical output was measured by using an optical spectrum analyzer. Fig. 5 shows the spectrum of the output signal collected through the waveguide from probe DNA labeled with Alexa Fluor 633. The emission signal is filtered by a band pass filter with cutoff at 660nm. The signal was measured before and after DNA attachment. There is a clear increase in the measured intensity due to the fluorescence signal from the attached probe DNA.

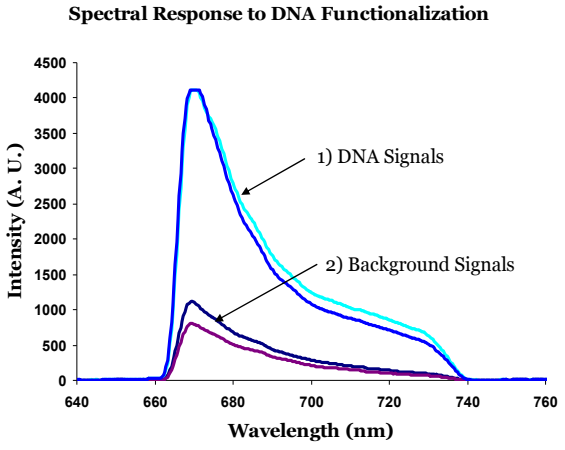


Figure 5: Spectra of optical emission signals received both 1) after introduction of fluorescent DNA and 2) when no fluorophores are present.

For the DNA hybridization experiments, the chitosan surface functionalized with probe DNA was exposed to matching or mismatching DNA solutions for 30 min at room temperature. In order to emphasize the selectivity, the mismatching DNA was twice as concentrated (8 μM) as the matching DNA (4 μM). Both DNA sequences were labeled at the 5' end with AlexaFluor 633 fluorophore. The response was analyzed with a fluorescence microscope and a spectrum analyzer.

Fig. 6 displays the intensity response to both matching and mismatching DNA sequences. A strong emission signal with a peak at about 650 nm is demonstrated only for the matching DNA sequence. The images on the right of the figure display the fluorescence microscope images of the sensor area after subjection to both the matching and mismatching DNA. These results were also demonstrated to be repeatable using the same device by washing away the target DNA with a 4 M urea solution at 95°C for 30 min and reintroducing the DNA samples.

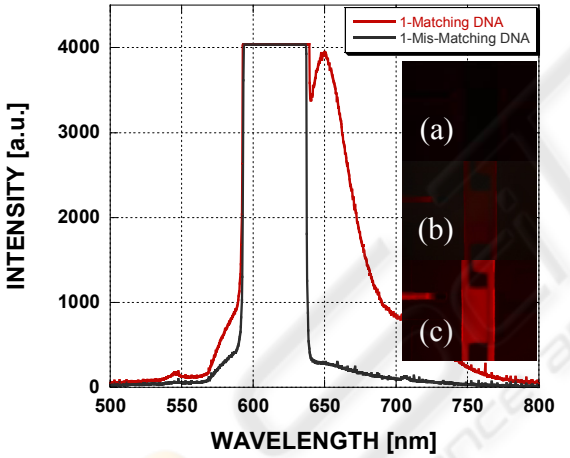


Figure 6: Collected output spectra demonstrating successful DNA hybridization. Inset: fluorescence microscope images of device: (a) after probe DNA attachment (b) after mismatching target DNA (c) after exposure to matching target DNA.

## 5 CONCLUSIONS

Chitosan enables a wide range of applications due to its unique structure and relative abundance in nature. We have reported the successful design, fabrication and testing of two distinct MEMS biosensors which utilize chitosan as a functionalization layer. Our microcantilever has a deposited film of chitosan on its surface to facilitate attachment of probe DNA. Its structure allows for highly sensitive detection due to mass loading. Our biophotonics sensor utilizes a novel sidewall pattern of chitosan to improve fluorescence emission capture into a planar waveguide. DNA hybridization experiments have been successfully performed with both devices. By bridging the world of MEMS with the world of biology through mechanical or optical detection, chitosan forms the missing link allowing for more robust and selective biological sensors.

## ACKNOWLEDGEMENTS

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