Surface stabilization of piezoelectric thin-film driven microcantilever for label-free DNA detection

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CONTENTS

List of figures

List of tables

국문 초록

DNA detection을 위한 압전 박막 캔틸레버 센서의 표면 안정화

바이오센서의 생체물질 검출 능력은 특이적 반응을 증진시키고 비특이적 반응을 억제함으로써 향상시킬 수 있다. 이를 위해 본 연구에서는 압전 박막 구동 캔틸레버의 표면을 퍼플루오로실란이라는 소수성 물질로 코팅하여 DNA의 비특이적 흡착을 최소화 함으로써 신뢰성 있는 캔틸레버 신호를 얻고자 하였다. 퍼플루오로실란층은 이소옥탄을 용매로 이용하였을 경우 짧은 시간의 반응으로도 캔틸레버 위에 안정적으로 형성되었다. 이를 이용하여 캔틸레버의 DNA 비감지표면은 퍼플루오로실란층으로 코팅하였으며 반대쪽의 DNA 감지표면은 자기조립 단분자층 형성을 위해 금막을 코팅하였다. DNA 검출에 있어서 10 μM probe DNA를 고정화하였을 경우 최적의 신호를 얻을 수 있었으며, 10 nM에서 5 μM까지의 target DNA를 적용하여 공진주파수의 변화를 살펴보았다. 또한 100 nM에서 0.7 μm, 1.0 μm의 두께를 갖는 캔틸레버를 이용하여 PZT 두께에 따른 공진주파수 변화도 살펴보았다. 이를 통하여 소수성 표면이 DNA 검출을 위한 캔틸레버 센서의 표면 안정화에 유용함을 알 수 있었고, 수십 nM 농도의 target DNA를 비표지로 검출함으로써 압전 박막 캔틸레버의 고성능 DNA 센서로서의 가능성을 확인할 수 있었다.

Key Word – 캔틸레버 바이오센서, 퍼플루오로실란, 비표지 DNA 검출, 표면안정화, 자기조립 단분자층,

Chapter 1 Introduction

1.1 Sensor based on microcantilever

Micro-Electro-Mechanical Systems (MEMS) is the integration of mechanical elements, sensors, actuators, and electronics on a common silicon substrate through micro-fabrication technology [1]. Over the last decade, a great deal of interest has been raised in applications of Microrectromechanical Systems (MEMS) for the detection of biological molecules and to the study of their forces of interaction. Due to advances in micromechanical systems, the mass production of extremely sensitive, low-cost, reliable sensors with high precision and improved response will be available. Bio-sensing with MEMS is based upon effecting a change in the dynamical properties of the device upon capture of a target analyte. To realize this, the device must be biofunctionalized in a manner such that the immobilization event induces a change in device response.

One of the most used structures in MEMS / NEMS devices is the cantilever [3-8]. A cantilever is, typically, a beam with a clamped edge and the other one free. The usual geometry for this structure is a rectangular shape. Based on the development of the atomic force microscope, the cantilever array technique monitors physical, chemical and biochemical processes taking place on the surface of the cantilever sensor. Recently, they have developed their structures and materials due to advances in the micro/nano electromechanical system (MEMS/NEMS), and have also

significantly expanded their applications due to the use of nano biotechnology. These biosensor application of MEMS technology remarked as biomedical or biological MEMS have become increasingly attention in a wide range of applications. A wide range of feasibility and diversity of BioMEMS device for biomolecules analysis have been continuously introduced and developed in the report by many groups within the last few years [2, 9-13].

When the sensor surface layer is exposed to an analyte, the cantilever mechanically responds by bending on the nanometer scale because of surface stress change, heat transfer, or mass changes. These surface stress sensors are based upon a direct measurement of the stress induced by the binding of a layer of ligands to an appropriately-prepared ("biofunctionalized") device surface [14-17]. Responses from the cantilever due to surface stress are induced by either a chemisorptions or physisorption phenomena. Reactions occurring at the cantilever surface can be measured directly without fluorescent or radioactive labels, and chemical reactions can be followed in real-time allowing analysis of reaction dynamics and determination of e.g. kinetic constants.

Hence, cantilevers can be used as mechanical transducers. They are very commonly used because of their versatility given that loads of different signals can affect their configurations, that is, can be sensed by means of a cantilever beam. Adsorption of molecules to the surface of the cantilever causes an increase (compressive stress) or a decrease (tensile stress) in surface area. Cantilevers can be used in two different modes of operation, i.e. static and dynamic [18-21]. In the static mode of operation, cantilever deflection is monitored continuously in order to detect

deformations produced by external measurands. Deformations in cantilever profile (static mode) can be produced by acceleration, mechanical surface stress and punctual force while changes in resonant frequency can be produced by mass addition and punctual forces [9, 22-23]. On the other hand, in dynamic mode changes in the value of the resonant frequency are measured. For excitation of resonance takes advantage of piezoelectric materials. Piezoelectric materials are referred to as 'smart materials' because of this internal material transduction process. These piezoelectric technologies provide a wide range of micro-devices that are sensitive, accurate, small, portable, robust, and have excellent characteristics. And another advantageous feature of using piezoelectric biosensor is that the same electromechanical transduction mechanism can be used not only for a sensing, but also for actuation. The piezoelectric sensing platform offers a very versatile technology base for the development of sensors, actuators, and smart structures. It is worth noting that most biological objects such as proteins and DNA are piezoelectrics, thereby using manmade piezoelectric materials could be very advantageous [24-25].

1.2 Research trends of microcantilever based DNA detection

Gerber's groups are firstly presented biomolecule interaction feasibility through DNA hybridization onto nanomechanical microcantilever surface. They monitored sensing the single stranded DNA hybridization with two microcantilevers in parallel which was functionalized with a selection of biomolecules [9]. The differential deflection of the cantilevers was found to provide a true molecular recognition signal

despite large nonspecific responses of individual cantilevers. Also, they report microarray of cantilevers to detect multiple unlabeled biomolecules and to quantitatively analyze DNA binding at nanomolar concentration within minutes [10]. Differential measurement including reference cantilevers on an array of eight sensors can sequence-specifically detect unlabeled target DNA.

In addition, Thundat group's cantilever-based biomolecule detection was focused on discrimination of DNA single-nucleotide mismatches [11]. They immobilized a 5'-thiol-modified probe on the Au-coated cantilever surface. The functionalized cantilever was then exposed to a solution containing complementary target ssDNA. They demonstrated that cantilever deflection varied with the complementary oligomer length. A change in deflection was caused by hybridization of a ssDNA probe (20-mer) complementary to the distal end of target ssDNA of four different lengths (9-, 10-, 15-, and 20-mers). They also could discriminate full and partial complementary sequences. For example, hybridization of 10-mer complementary target resulted in net positive deflection, whereas hybridization with sequences containing one or two internal mismatches resulted in net negative deflection.

Majumdar group are applied to DNA detection at cantilever, according to ssDNA length and density focused to difference of surface stress [12]. The results of this study report that due to the phosphate backbone (net negative charge) repulsion interactions between the single-stranded DNA (ssDNA) molecules immobilized on the surface of the cantilever induce a compressive stress, bending the cantilever downwards. After the complementary DNA strands and hybridization occurs, the cantilever bends upwards, releasing the compressive stress generated in the first step.

Upon immobilization, each ssDNA is forced to occupy a region of space and to minimize the clustering effect the ssDNA forces the cantilever to bend downwards thereby increasing the available surface area. After hybridization, the cantilever bends upwards since double-stranded DNA (dsDNA) requires a smaller region of space due to it more rigid and compact structure. Cantilever motion is created because of the interplay between changes in configurational entropy and intermolecular energenics induced by specific biomolecular reactions. The surface stress was observed to be compressive resulting in a downward bend of the cantilever.

Mutharasan's groups are PEMC (Piezoelectric Excited Millimeter-sized Cantilever)-based macro-sensors were functionalized with 15mer single stranded DNA and exposed to 10mer complementary sequence at concentrations of 1 fM, 1 pM, and 1 μM [13]. The majority of the methods reported for direct detection of DNA targets are at micromolar to nanomolar range and very rarely lower than picomolar. They show for the first time successful detection of small DNA sequences at femtomolar concentration both in buffer and in serum.

As a result, current work in this field is focused on maximizing sensitivity and selectivity about the specific molecules, reducing the non-specific interaction [9-13]. However, for these reasons mentioned above, the other research groups have studied detection of raw DNA focusd on cantilever behavior with overlooking surface stabilization.

Table 1 Research trend of raw DNA detection

Group	Cantilever dimension	Measurement environment	Detection method	Detection limit	DNA length
Majumdar	20 x 200 x 0.5 µm	Liquid	Optical (CCD)	-	Probe: 20mer Target:20mer
Thundat	180 µm (L) x 1 µm (t)	Liquid	Optical (PSD)	-	Probe: 25mer Target:10mer
Gerber	100 x 500 x 250 µm	Liquid	Optical (PSD)	75 nM	Probe: 12mer Target:12mer
Mutharasan	4 x 1.8 mm x 127µm	Liquid	Electrical	1 fM	Probe: 15mer Target:10mer

1.3 Research objective

The essential abilities of biosensor are sensitivity, selectivity and specificity. Being differently from other biosensors, the cantilevers have the mechanical structure, which is composed of the recognition layer on one side and the non-recognition layer on the other side. On account of this aspect, the cantilevers are facile to be influenced by non-specific binding of target or environmental biomolecules. If non-specific binding on the microcantilever surface happens, it may disturb the measurement of the resonant frequency due to signal distortion. Therefore, the surface stabilization of microcantilever is essential to acquire the reliable resonant frequency. In this thesis, to stabilize the surface and to minimize the nonspecific binding of target DNA, the microcantilevers were coated with perfluoro-silane, and its performance was analyzed by contact angle analyzer, XPS and so on. Based on the reliable surface, the resonant frequency shifts of the cantilever were measured as a function of the concentration between 10 nM and 5 μM. Moreover, a tendency of signals according to the concentrations was explained theoretically.

Chapter 2 Experiment

2.1 The fabrication of microcantilever

The actuating layer (PZT) embedded cantilevers are fabricated by the surface micromachining. To make a self-actuating cantilever, already referred, the well known piezoelectric PZT (52/48) actuating layers are embedded in microcantilever. In the thesis, actuating layer (PZT) of the cantilevers allows electrical detection of the resonant frequency shift of a fine difference caused by DNA hybridization on the functionalized microcantilever surface. Our piezoelectric microcantilevers are consisted of multi-layers of Ta/Pt/PZT/Pt/SiO_2 on a silicon nitride supporting layer. As shown in Figure 2.1., the substrates to form PZT capacitors were 100mm (4 inch)-diameter p-doped Si (100) wafer covered with 1.0 μm thick low stress silicon nitride deposited by Low Pressure Chemical Vapor Deposition (LPCVD). The layer of silicon nitride plays important roles in not only supporting the beam-shaped structure, but also enhancing the elastic performance of self-actuating cantilever; by the higher elastic performance of Silicon Nitride than Silicon. The bottom electrode was prepared by sputtering a thin Ta (0.03 μm) adhesive layer followed by a Pt layer with thickness of 0.15 μm. The dielectric structure of piezoelectric material between the platinum (Pt) layers provides the actuating force through the piezoelectric and converse piezoelectric effect. After that, the PZT thin films were deposited on the bottom electrode by the spin coating of mixed PZT solution (Diol-based sol-gel route,

0.3M) at 3000 rpm for 30 sec. They have been fired at 400 °C for 5 min and then annealed at 650 °C for 10 min. For a metal-ferroelectric-metal (MFM) capacitor structure, a Pt layer was deposited onto a PZT film as a top electrode via RF sputtering. After all layers were formed, the top-down method was used in micro-fabrication.

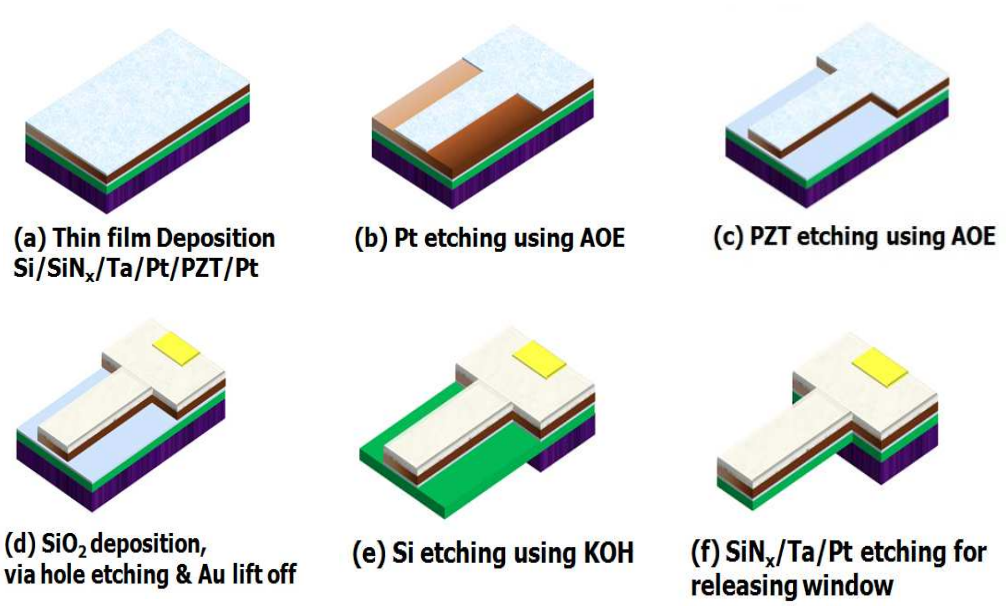


Figure 2.1. The scheme of micro-fabricated process

The actuating layer embedded microcantilevers are fabricated within six main steps. First, from the PZT layer to the Pt layer which is a top electrode were etched by Advanced Oxide Etcher (AOE) that is a one of the inductive coupled plasma (ICP) etching method. A SiO_2 thin film with a thickness of 0.2 μm was deposited onto the

top electrode via a plasma-enhanced chemical vapor deposition (PECVD) process. To prevent the physical and/or chemical denaturalization of piezoelectric material (PZT) at the biological surface treatment process, the SiO_2 layer was deposited as passivation layer between top and contact electrode. Following this, contact holes on the top electrode were etched. The contact electrode and the top electrode were formed in the SiO_2 layer by evaporation and lift-off process. After that, backside silicon nitride window was patterned with RIE. The bulk silicon (525 ± 20 μm) was then etched using KOH silicon wet etchant. Finally, the bottom electrode of Pt layer and the residual silicon nitride layer were respectively etched by AOE and RIE for making a free standing cantilever [26].

2.2 Device packaging

As the development of microelectronics is still driving towards further miniaturization of new materials, processes and technologies are crucial for the realization of future cost effective micro-systems and components. For reliable and low cost assembly for such applications, new placement in joining technologies are demanded as today's packaging technologies only allow the assembly of those small dies and components with a very high effort with high cost. With ongoing miniaturization, the protection of the microsystems are required by a polymer needs to be decreased in thickness, yet providing maximum protection. Here, besides mechanical stability, humidity barrier functionality is a key factor for system reliability. The development of MEMS devices has come a long way and new

boundaries have been reached. A problem still remains, however, in being able to communicate efficiently with these devices. While MEMS sensors are small, the packages that contain them are considerable in size to facilitate their handling and assembly [27].

First, in order to package, we have to bond between cantilever device and ceramic substrate with epoxy. And then cure at 150 ℃ for 5 min. The device packaging was determined after perfluoro-silane passivation treatment on the SiO_2 surface of microcantilever. The 4 ea of bottom electrode and the 12 ea of upper electrode for each device were wire bonded using wire bonder [WEST BOND, 4700E]. To prevent wire attack and a short circuit, keep in PDMS to wire on cantilever device curing at 80 °C for 20 min on hotplate. At the backside, lock up metal cap using epoxy even as bioassay solutions do not soak its way. The metal cap should be parylene coated 0.5 μm for passivation. And then, metal cap is bonded at ceramic substrate using epoxy, then cure at 150 ℃ for 10 min.

2.3 DNA detection for microcantilever-based self-actuating sensor

As one of most powerful tools for gene investigation, DNA microarray technology has shown significant increase in use after its invention approximately twenty years ago. Unfortunately, the fluorescence signal based detection used in traditional DNA microarrays necessitates complex and delicate laser scanners and specialized software making wide accessibility impossible; thus, this is the most important merit required by personalized medicine. [28]

Now, we report a microcantilever-based DNA assay used for the detection of 27-mer target DNA. The microcantilever detection approach has attracted considerable attention as a means of label-free detection of biomolecules in recent years [29]. While the majority of currently used detection schemes are reliant on biomarkers, such as fluorescent labels, time, effort, and chemical activity could be saved by developing an ultrasensitive method of label-free detection [16]. In our group, the label-free detection is already confirmed. But, there is another important point as crucial as label-free; surface stabilization. Our object is the specific biomolecular binding between probe DNA and target DNA on the surface of a microcantilever beam results in resonant frequency shift of microcantilever. A promising approach for detecting biomolecules follows their binding to immobilized probe molecules in microfabricated cantilevers; binding cause surface stresses. The surface chemistries used in functionalization of MEMS and NEMS sensors give the devices their capability for sensing. Coated with probe DNA layers which have specificity to a complementary target DNA, sensor signals can then be attributed to target DNA detection [29].

In the thesis, a hybridization method in which the probe DNA are directly hybridized with the target DNA was validated by the alteration of cantilever's dimension and actuating layer (PZT) thickness at optimized the experimental conditions,.

2.3.1 Material for the DNA sensing

The thiolated probe DNA for the binding on the gold surface of the microcantilever and the fluorescence modified target DNA and target DNA-1 were purchased from Bioneer Inc. (Seoul, Korea). And HPLC grade; over the 1 O.D. guaranteed. Poly-C chain, which consists of 24 carbons, is attached the 5' end of probe DNA. It makes probe DNA longer than spacer thus hybridization efficiency between probe DNA and target DNA is increased. The thiolated spacer was added after immobilizing the probe DNA in order to control the immobilization and DNA activation [30]. The thiolated spacer was supported to from Konkuk University, professor Yeo Woon Seok.

Target DNA was designed as a complementary sequence of Probe DNA and all targets DNA was terminal Cy3-modified for fluorescence confirmation. Also, target DNA-1 was designed as non-specific sequence 25 mer for control experiment. The details are shown in Table 2.

Table2. Design of DNA sequence

Perfluoro - silane	**1H,1H,2H,2H-Perfluorodecyltriethoxysilane**	**10 mM**
Probe DNA	**5'- HS - $(CH_2)_{18}$ -T_{10}CTT TCC TTC TAT TCG AGA TCT CCT CGA - 3'**	**10 µM**
Spacer	**HSC_{11}-EG_3-OH**	**5 mM**
Target DNA	**5' - Cy3 - TCG AGG AGA TCT CGA ATA GAA GGA AAG - 3'**	**10 nM -5 µM**
Target DNA-1	**5' - Cy3 - AAT GAA GGG TGG GTC CAC CGG TCT A - 3'**	**10 nM -5 µM**

2.3.2 Surface functionalization

Our object is the specific biomolecular binding between probe DNA and target DNA on the surface of a microcantilever which makes resonant frequency shift of microcantilever. As biosensors, the detection specificity is one of the most important factors for their performance. To achieve this, the cantilever-based biosensors non-recognition layer should have the inert layer.

We choose PEG (2-[methoxy(polyethyleneoxy)propyl] trimethoxysilane, Gelest, Inc, USA) and Perfluoro-silane (1H, 1H, 2H, 2H- Perfluorodecyltriethoxysilane) as possible passivation material candidates. Also, passivation solvent candidates are Ethanol (99.9%, DC chemical Co.), Chloroform (99%, CARLO ERBA Co.), and Iso-octane (99%, Wako Co.).

At first experiment, 1mM of PEG was used. The PEG solution was diluted in 99.9% Ethanol to a concentration of 10 mM. The microcantilevers were dipped in a 10 mM PEG solution for 6 hours and then cured at 150 ℃ to deposit the PEG on the SiO_2 layer on the cantilever surface [31].

Second, the SiO_2 surface of microcantilevers was passivated with perfluoro-silane in chloroform, toluene, iso-octane layer prior to the gold film evaporation and the probe DNA immobilization. Before DNA immobilization control, non-specific binding at cantilever's front side is a major problem that can affect the unexpected noise components. In other research groups, a perfluoro-silane layer was deposited on the other side of the microcantilever [32]. Perfluoro-silane, the well-known organosilane repellent was polymerized in the cantilever surface. In order to make an

inert layer, plasma treatment was performed for 1 min. Perfluoro-silane deposited on the SiO_2 for 30 min, which blocked the chemical interaction or physical adsorption due to its inertness. Washing has followed solvent (Iso-octane, chloroform, toluene), acetone, ethanol. After treatment for 30 min, then cure at 150 ℃ for 10 min. This process will be made the cantilever surface inert, stable, and reproducible.

2.3.3 Probe DNA immobilization

The concept of DNA biosensors is sustained by the need for rapid and highly sensitive, analytical tools for genetic detection. Their implementation is based on three key steps.

(i) Immobilization of single stranded probe DNA onto a substrate;

(ii) Single stranded target DNA hybridization

(iii) Read out

These steps involve complementary knowledge in various fields such as surface physics and chemistry, molecular electrochemistry, micro-technologies. According to this step, the immobilization of DNA strands is an essential step in the development of any DNA biosensor. The microcantilevers were used for this DNA assays, and probe DNA were designed, which can be applied to the surface of cantilever-based DNA sensors [33]. Fabricated actuating layer embedded sensors based on resonating silicon nitride cantilevers coated on backside with gold. Cr/Au layers (100/500 Å) were deposited on the other side of the microcantilever by an e-beam evaporator, on which the probe DNA can be readily immobilized.

The probe DNA with 5' terminal thiol was diluted in the TE buffer (pH 7.4, Bioneer Inc.) to make a 10 μM concentration. The 10 μM probe DNA was immobilized on the freshly Au-coated microcantilevers for 3 hours at room temperature. The thiolated probe was used, which was immobilized directly on the Au surface with high efficiency. Then, the thiolated spacer in ethanol was immobilized for 90 minutes, which organize the probe DNA for activation and density control in DNA hybridization. This results in a better accessibility of the surface-confined probes towards their target. This immobilization approach is performed frequently in combination with alkanethiol "backfilling" molecules. For making well mixed DNA/alkanethiol films, using the C_{11} spacer was found to be more densely packed than C_6 spacer [34-35]. The probe DNA and spacer immobilized cantilevers were washed with ethanol and stabilized in TE buffer for 30 min. The immobilization method provides a probe DNA with uniform density on gold surface for efficient binding of spacer and essentially overcomes the poor efficiency caused by heterogeneous coupling between the solid bodies though DNA hybridization.

2.3.4 Hybridization with target DNA

To quantitative analyze the target DNA concentration and the dependence of the dimensions of the microcantilever on the minimum detectable sensitivity; we are fixed to Probe concentration of 10 μM, which have two dimensions, 50 μm x 150 μm (width x length), and 30 μm x 90 μm (width x length) with complementary sequence target DNA hybridization at 25 °C and room humidity for 1 hour.

Figure 2.2. shows schematic of probe DNA immobilization and target DNA interaction on the microcantilever surface. The microcantilever was Figure 2.3(a). shows good hybridization efficiency in TE buffer contained 1 M sodium chloride at room temperature [36]. It demonstrates similar hybridization performance into TE buffer contained 1 M sodium chloride and surrounding melting temperature of target DNA. It supposed that 1 M sodium chloride is activating DNA hybridization. So, all experiment that hybridization steps is progressed TE buffer contained 1 M sodium chloride at room temperature. In this step, the target DNA in TE contained 1M sodium citrate from 10 nM to 5 μM were applied to the microcantilever treated with 10 μM probe DNA to check the correlation between the concentration of probe DNA and resonant frequency shift of cantilever. In this time, hybridization method was chosen flowing target as shown in Figure 2.3(b).

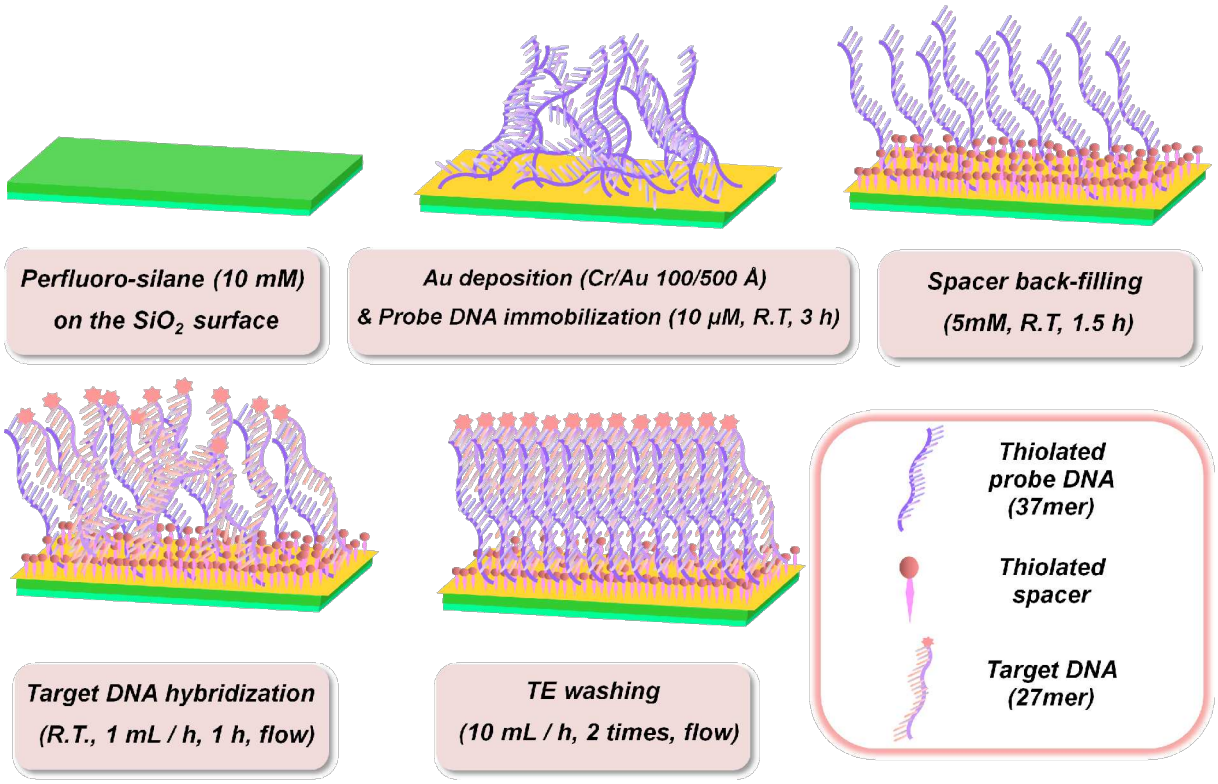
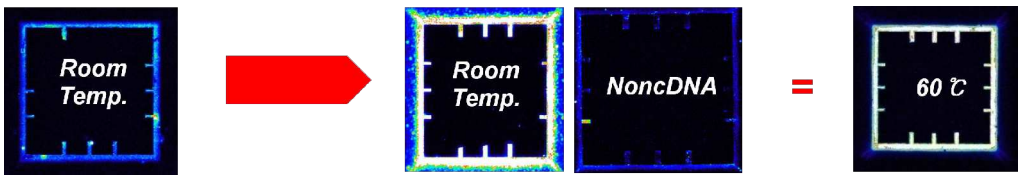


Figure 2.2. The illustration of the microcantilever-based DNA detection

(a)



(b)

Figure 2.3. (a) Hybridized fluorescence images of cantilever device according to hybridization temperature (b) Hybridization chamber

The binding flow rate was 1 mL / h for 1 hour. To minimize non-specific binding, the washing flow rate was 10 mL / h for 18 minutes at 3 times. To confirm the specific binding after the probe - target DNA interaction, we performed fluorescence labeling using FluoroLinker Cy3 Mono Reactive Dye. With the help of this method, direct hybridization between surfaces immobilized probe DNA and target DNA, the reliability of this method was demonstrated by hybridization experiments using these assays.

At the experiment, major experiment and negative control experiment was performed in parallel. In detail, the selectivity of this method was confirmed by fluorescence and resonant frequency shift through the detection of a non-

complementary sequence target DNA and TE buffer. In addition, to examine that resonant frequency shift has caused by DNA interaction only, we developed spacer backfilling on the microcantilever. Furthermore, to evaluate the thickness effect of cantilever on the sensitivity, the cantilevers with different thickness (PZT thickness: 0.7, 1.0 μm) were applied to the direct DNA hybridization.

2.4 Measurement

For direct methods, the most common tool is the use of a PZT, our cantilever system which is capable of vibrating at different frequencies very accurately depending on the driving voltage. Using the same procedure that was used in the direct hybridization experiment, the resonance frequency was measured before and after hybridization with the immobilized probe DNA and the target DNA hybridization. The optimized temperature and humidity maintenance are essential during DNA detection. In order to optimized measurement condition, the probe DNA (10 μM) immobilized cantilever was treated directly with the target DNA at 10 μM concentration. We examined resonant frequency shift as function of humidity as shown in Figure 2.4. The result of experiment has confirmed saturated resonant frequency shift at 50% humidity. For more stable measurement condition, all experiments was carried out at 33 °C and R.H. 70% using MiCan (Cantis, KOREA). There are eight devices (96 microcantilevers) on the fabricated integrated system for microcantilever-based DNA detection at one time. The system has small chamber to keep the same environment within resonant frequency measurement. And all the

hybridization steps were confirmed by the fluorescence image of the back side view. After the probe-target DNA interaction, confocal microscope (Carl Zeiss Co.) was used to observe the fluorescent scanning image. This method with compared to conventional methods for detecting DNA hybridization, electrical transduction is the goal of numerous research works due to low cost and power requirement, portability, independence of sample turbidity, ease of miniaturization and compatibility with manufacturing nanotechnologies. Figure 2.5. shows temperature and humidity controlled small chamber.

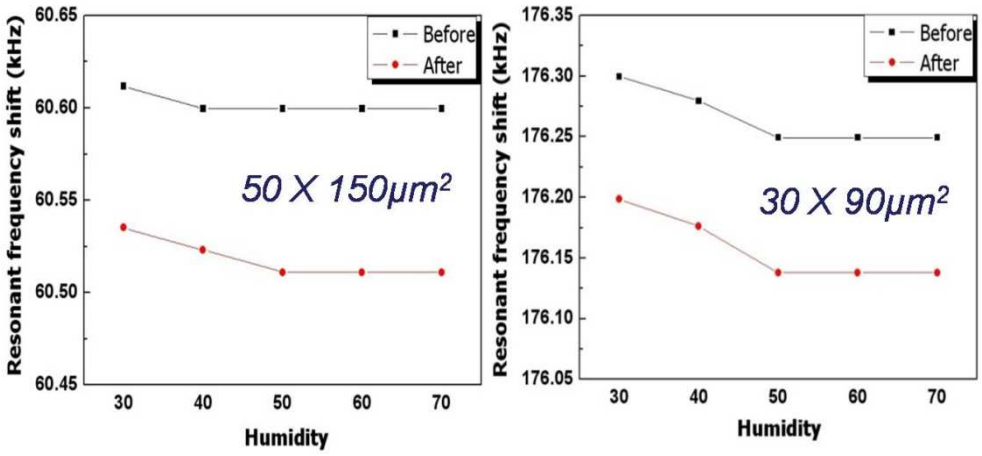


Figure 2.4. Resonant frequency shift as function of humidity according to cantilever dimensions

Figure 2.5. Measurement humidity controlled chamber

Chapter 3 Result and Discussion

3.1 The fabrication of microcantilever

The piezoelectric microcantilevers consist of multiple layers such as SiNx/Ta/Pt/PZT/Pt/SiO_2 for self-actuation using piezoelectric and reverse-piezoelectric effects. Through the micro-fabricated process, two different dimensions of rectangular shaped microcantilevers were fabricated: dimensions of 50 μm x 150 μm x 2.48 μm and 30 μm x 90 μm x 2.48 μm (width × length × thickness). Unit device has twelve microcantilevers that consist of six microcantilevers with dimension of 50 μm x 150 μm (width x length) and six microcantilevers with dimension of 30 μm x 90 μm (width x length) that can increase the accuracy of the bioassay results due to different dynamic ranges and signal change variances. The important point of device is to realize microcantilever arrays for enhanced of reliability and accuracy.

SEM images of microfabricated cantilevers were presented as shown in Figure 3.1.

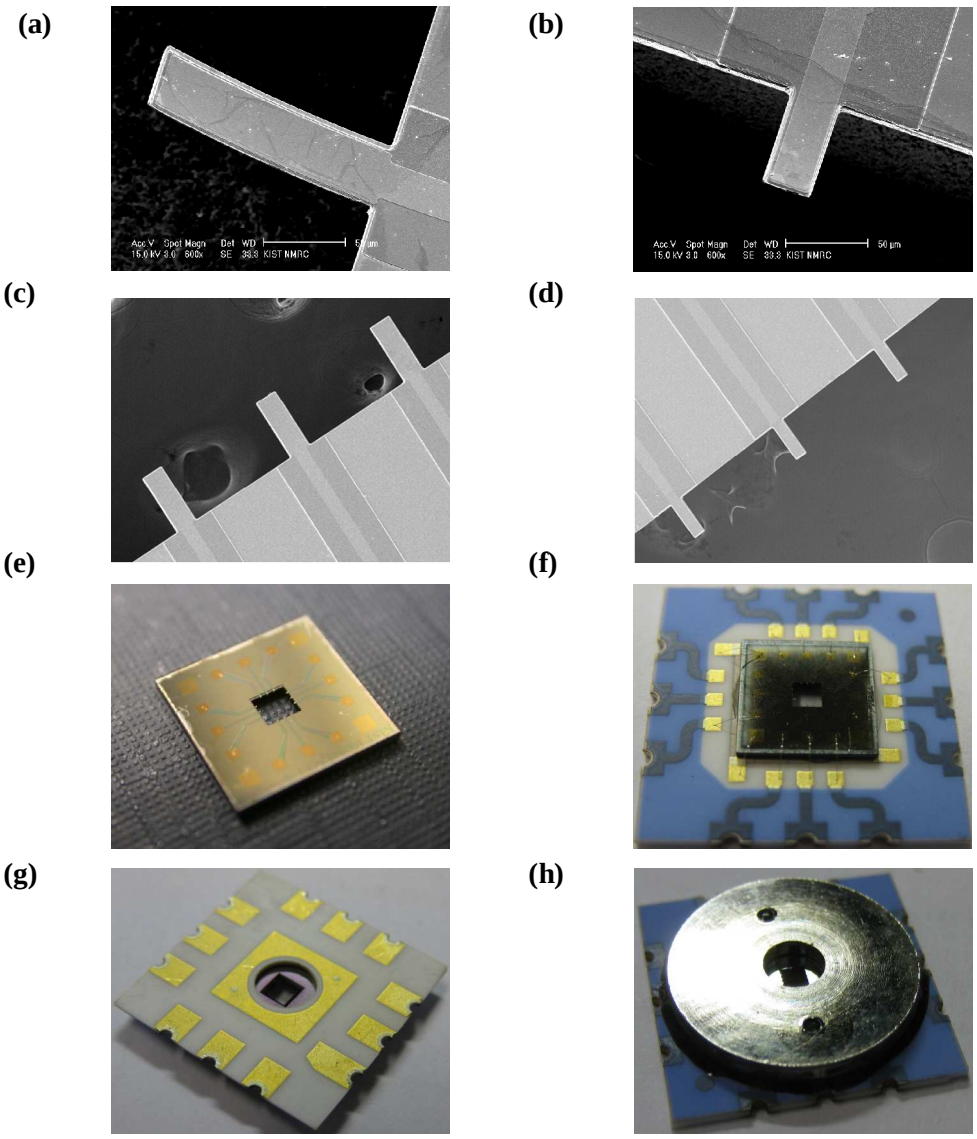


Figure 3.1. Photographs of microfabricated device and SEM images of different dimensions of microcantilevers (a,b) Single cantilever device with dimension of 50 µm × 150 µm and 30 µm × 90 µm (width x length) (c,d) Cantilever array (e) Unit device consisted in twelve microcantilevers (f) Wire bonded unit cantilever device (g,h) Front side and back side of packaged cantilever device

3.2 Surface functionalization

The appropriate controls of background signals for subtraction are often necessary to void non-specific effect and produce biosensors with meaningful results. Although DNA-DNA hybridization is highly specific, non-specific physisorption or cross-reactivity of the receptor with another biomolecules are still present. Thus, non specific binding is a very important issue that affects all types of binding assays.

Hence, the goal of surface stabilization is minimizing nonspecific binding and environmental effect such as humidity and temperature. First, PEG-treated SiO_2 surface didn't show the non-specific binding of DNA seriously in the fluorescent analysis. However, in the case of resonant frequency is occurred signal distortion. We estimate that resonant frequency shift is due to water absorption by water-compatible PEG layer, not due to DNA hybridization. Hence, we confirmed that this passivation system was not suitable for our nanomechanical system. In the next, the cantilevers were covered with perfluoro-silane layer. The optimal solvent was chosen. The perfluorination in chloroform for 3 hours was not effective. However, the perfluorination in iso-octane just for 30 minute was very effective for our cantilever. The surface property maintain over 1 month. Furthermore, we evaluated the stability of perfluorinated surface under various experimental conditions (TE buffer, Ethanol, probe DNA, spacer, target DNA). The result of surface stabilization measured by contact angle analysis experiment (Contact angle analyzer – SEO, phoenix 300) shows as shown in Figure 3.2. The non-specific binding of the perfluoro-silane layer was confirmed by fluorescent imaging. And then, the level of non-specific binding of

the other side was reduced remarkably. Therefore, environmental condition effect occurred in has less hydrophobic surface than hydrophillic surface in our systems.

In the next step, chemical compositions and inert layer thickness of perfluoronated surface were analyzed by XPS (G.J. SCIENTIFIC CO.). The result of XPS analysis has shown in Figure 3.3. In regard with XPS analysis, sputter rate is 1 nm/0.1 min. Based on this analysis, I found that thickness of perfluoronated layer is about 1 nm.

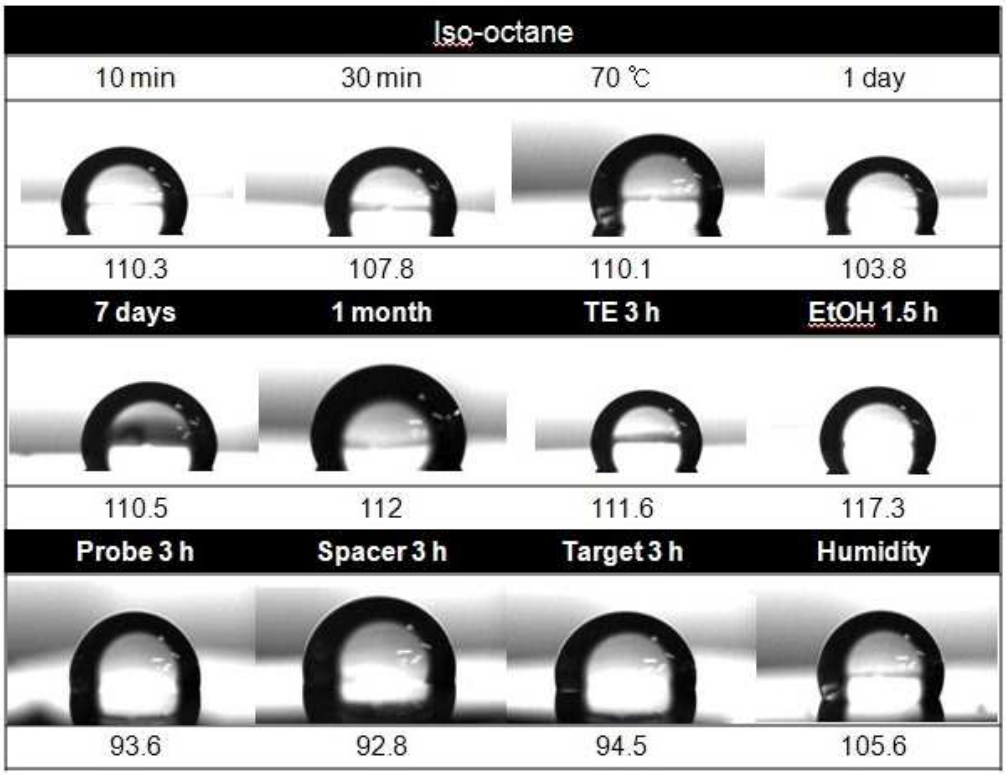
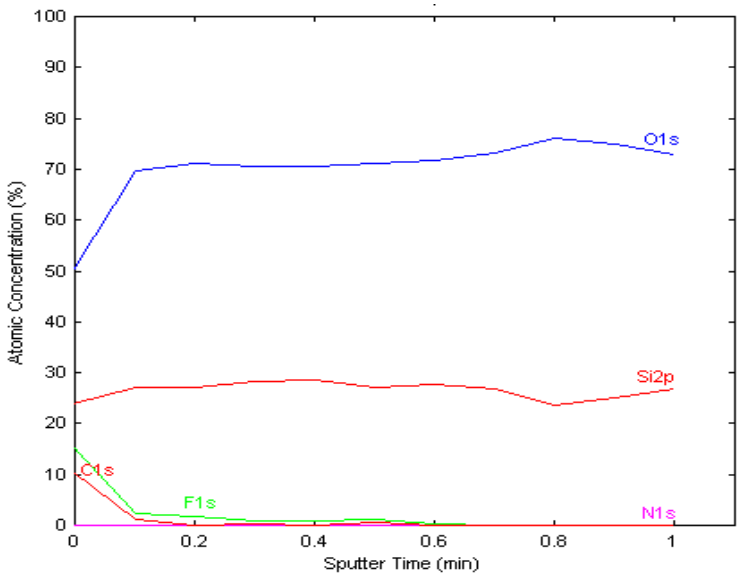


Figure 3.2. Contact angle images of passivation layer treated SiO_2 surface

(a)



(b)

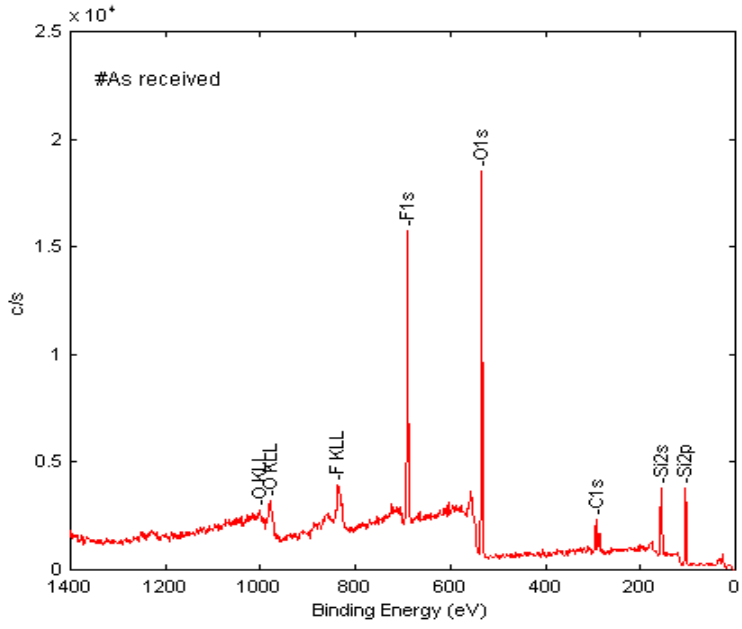


Figure 3.3. XPS data (a) Depth profile of perfluoronated SiO_2 surface

(b) Component parts analysis of SiO_2 surface

3.3 Hybridization with target DNA

First, to evaluate the relationship between the target DNA concentrations and the resonant frequency shifts, two types of cantilevers of different dimensions (30 x 90 μm^2 and 50 x 150 μm^2) with 1 μm PZT layer were evaluated with increasing the concentration of the target DNA. As mentioned above, at concentration of probe DNA (10 μM), the resonant frequency shifts of cantilever were stable enough to be applied for further DNA detection. Assuming that the hybridization between the probe DNA and target DNA has a quantitative relationship, the probe DNA immobilized on the cantilever was treated with the target DNA at different concentrations. Figure 3.4 shows experimental resonant frequency shifts as a function of 10 nM ~ 5 μM target DNA concentration, which also indicates that the detection sensitivity depends on the dimension of cantilever. In detail, the resonant frequency shifts due to the specific binding of target DNA with its specific probe DNA on microcantilever surface is 36 Hz for the 50 μm x 150 μm cantilevers and 178 Hz for 30 μm x 90 μm cantilevers at the concentration of 100 nM. These results match the well-known fact that the cantilever structure is more sensitive when the size is smaller [37].

The resonant frequency shifts increased according to hybridization between the probe DNA and target DNA from 10 nM to 100 nM. The results clearly indicate that the resonant frequency shifts are proportional to the target DNA concentrations. Resonant frequency shifts for the target DNA of 10 nM, 50 nM, and 100 nM are 12, 114 and 178 Hz, respectively. We estimate the resonant frequency shifts are originated from the released surface stress during the probe DNA and target DNA

binding process. This trend matches with that of the surface stress obtained by Wu et al [21]. Furthermore, some papers analyzed theoretically about the stress and mass effect to the resonant frequency shifts, and these papers conclude that stress effect is more dominant than the mass effect [18, 19, 20]. Therefore, the major driving force of the resonant frequency shifts must be due to the surface stress released by DNA hybridization. In addition, the fluorescence images are reliable with the resonant frequency shifts according to the target DNA concentration. All the hybridization steps were confirmed by the fluorescence image of the backside view, which shows a uniform distribution of Cy3 labeled target DNA on the cantilever surfaces, as shown in Figure 3.6. The fluorescence images of the microcantilevers were used to be compared with the results of the resonant frequency shift in a controlled environment. Non-specific binding of target DNA on the front-side of cantilever was not observed. Moreover, although not marked in the plot, there were barely changes in the control signal. We predicted that resonant frequency shifts are proportional to the high concentrations of the target DNA. However, experimental result shows that the resonant frequency shifts gradually decreased as the concentration increased from 300 nM to 5 μM. It is obvious that much more interaction occur when target DNA at high concentration hybridize with probe on the cantilever surface. The hybridization at high concentration may induce the surface stress because the narrow space between double stranded DNA increase the repulsive force.

In the next, the resonant frequency of 100% spacer-filled cantilevers was measured in varying humidity condition. As a result, we confirmed the resonant frequency shifts are barely changes as shown in Figure 3.7. Therefore, the output signals of the

cantilever sensor appear to reflect the concentration of target DNA. In such a configuration, a micro-cantilever, selectively modified at one side by a specific probe DNA, quantitatively responds to hybridization through mechanical vibrating due to a change in surface stress. As mentioned earlier, direct transduction relies on the measurement of physicochemical changes occurring at the recognition layer induced by hybridization.

As shown in Fig. 3.5., the resonant frequency was shifted by the 1.0 μm PZT embedded microcantilever device that was clearly distinguishable from the case of the 0.7 μm PZT embedded microcantilever device at 30 x 90 μm^2.

The resonant frequency change due to the complementary target DNA hybridization to its specific probe DNA on microcantilever surface is determined as 178 Hz, 126 Hz after hybridization 100 nM target DNA at 1.0 μm, 0.7 μm, respectively. This experiment is to find out a suitable thickness for good actuating performance and stable resonant frequency. As a result, the actuating performances of 1.0 μm PZT cantilever are better than those of 0.7 μm PZT cantilever. In other words, slightly thicker cantilevers were more sensitive than thinner cantilevers

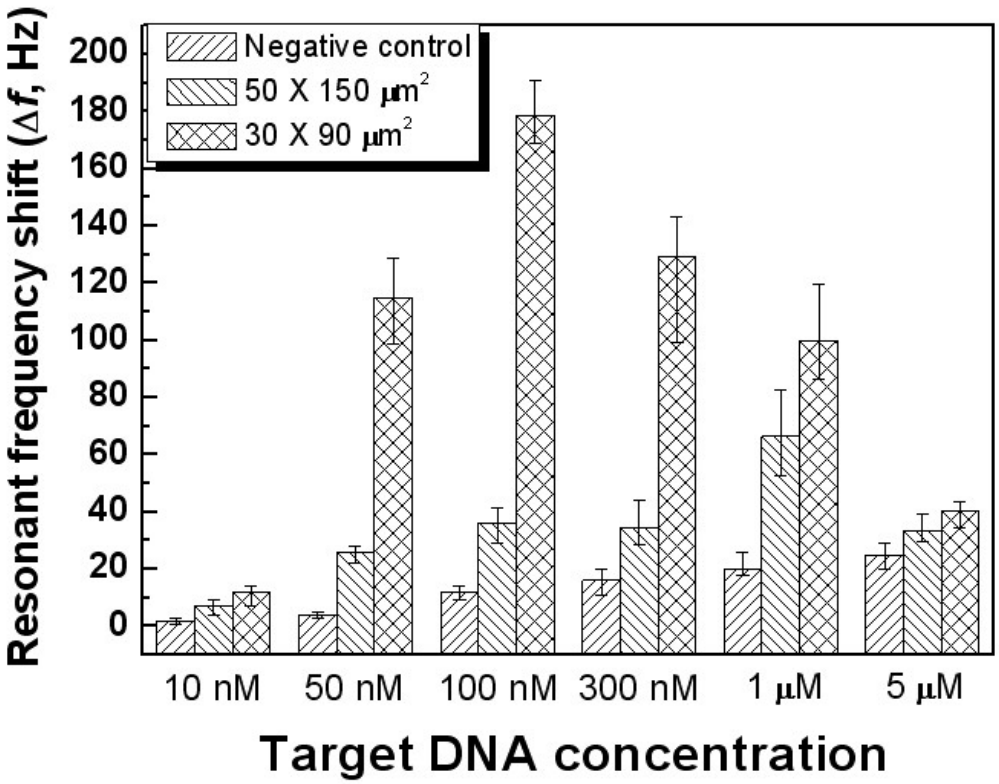


Figure 3.4. The result of microcantilever-based DNA hybridization with different cantilever dimensions and a various of target DNA concentration

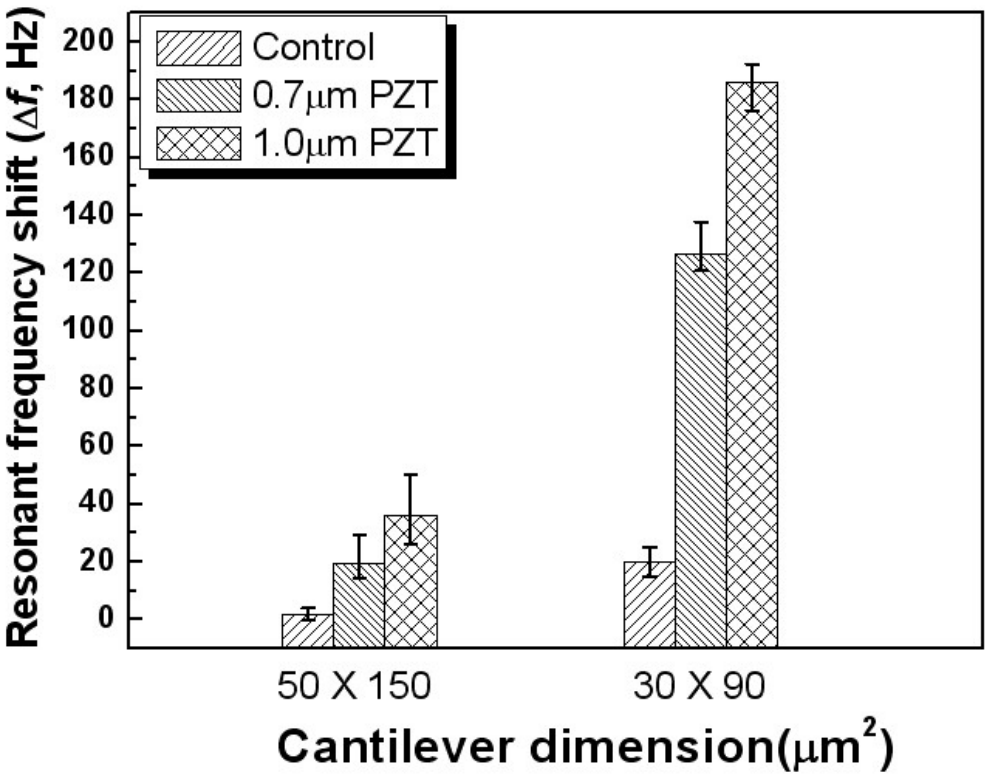
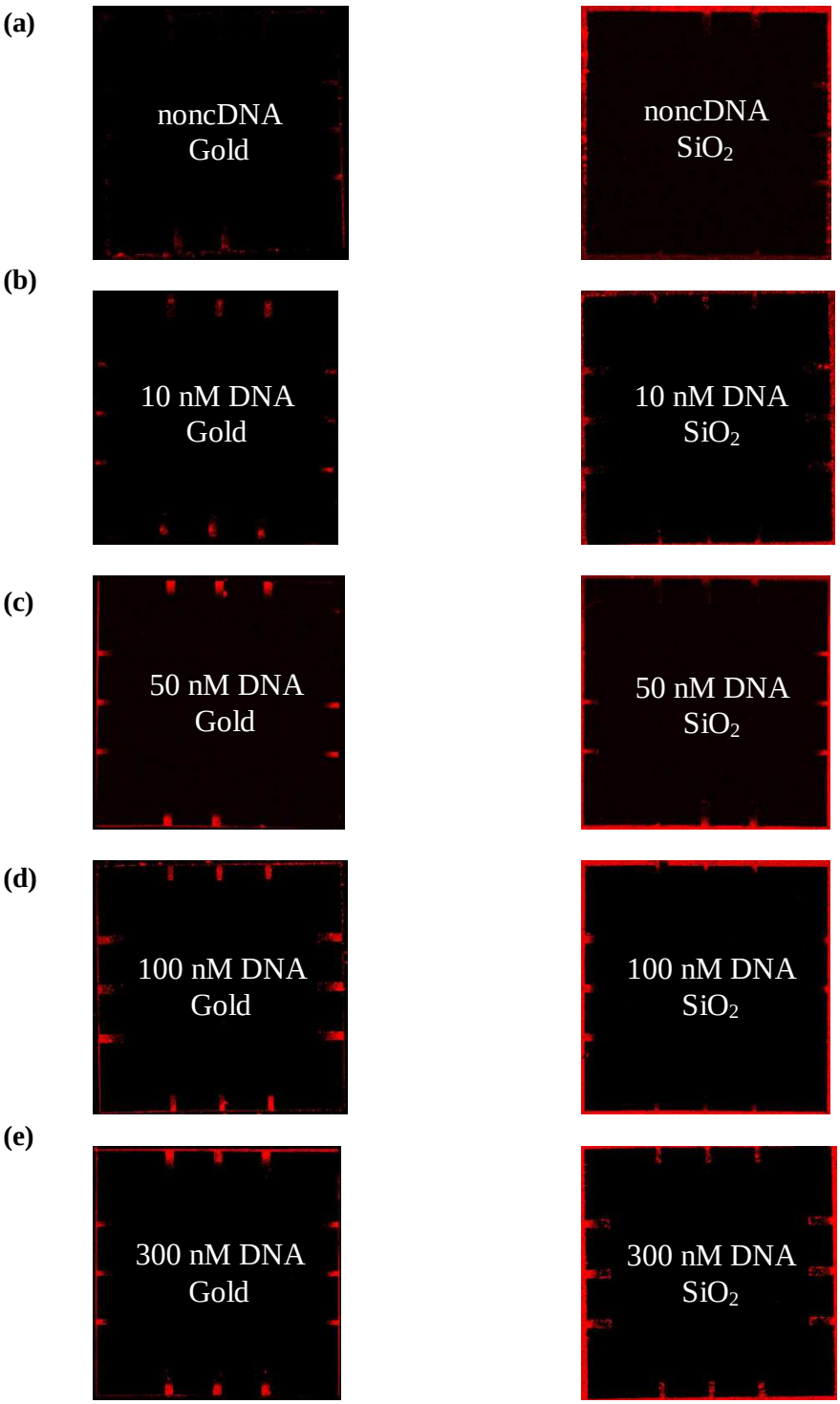


Figure 3.5. Experimental resonant frequency changes as a funtion of target DNA concentration, which clearly indicates that the actuating layer thickness of cantilever


(a)
noncDNA
Gold
noncDNA
SiO2
(b)
10 nM DNA
Gold
10 nM DNA
SiO2
(c)
50 nM DNA
Gold
50 nM DNA
SiO2
(d)
100 nM DNA
Gold
100 nM DNA
SiO2
(e)
300 nM DNA
Gold
300 nM DNA
SiO2

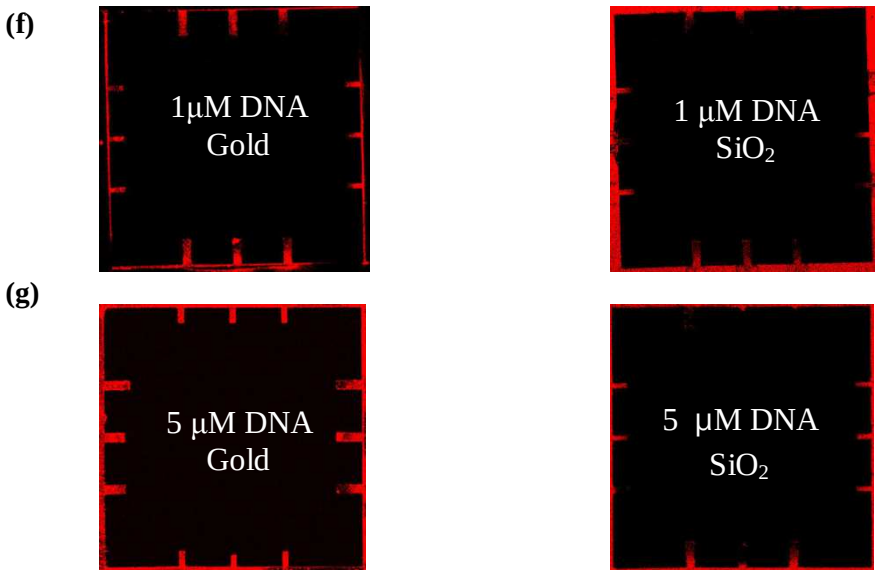


Figure 3.6. Fluorescence image with several of target DNA concentrations

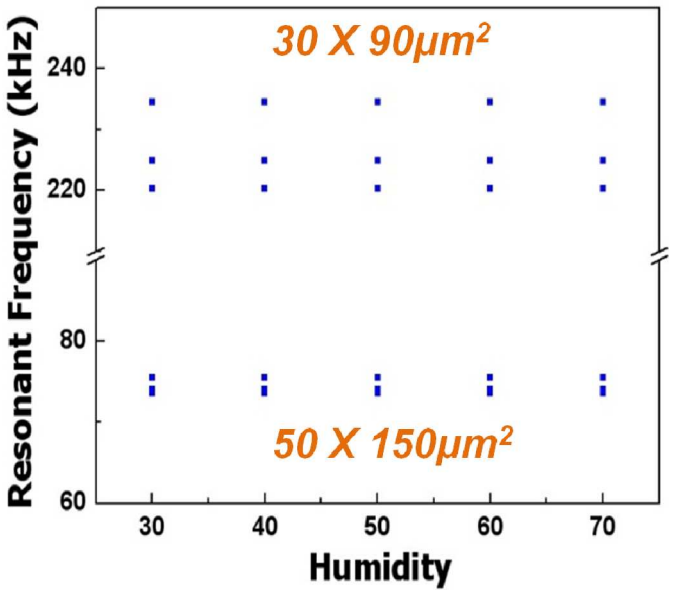


Figure 3.7. Experiment on the humidity effect for 100% PEG spacer-filled cantilever

Chapter 4 Conclusions

The essential abilities of biosensor are sensitivity, selectivity and specificity. Being differently from other biosensors, the cantilevers have the mechanical structure, which is composed of the recognition layer on one side and the non-recognition layer on the other side. On account of this aspect, the cantilevers are facile to be influenced by non-specific binding of target or environmental biomolecules. In this thesis, to stabilize the surface and to minimize the nonspecific binding of target DNA, the microcantilevers were coated with perfluoro-silane. The optimal reaction condition is determined as dipping the cantilevers in isooctane at room temperature for 30 minutes. The surface stability under various conditions and the thickness of perfluoro-silane were confirmed by contact angle analyzer and XPS respectively. Based on the reliable surface, the resonant frequency shifts of the cantilever were measured as a function of the concentration between 10 nM and 5 μM. The resonant frequency shifts were increased from 10 nM to 100 nM, however they decreased up to 5 μM concentration. A tendency of signals according to the concentrations was explained as the release of surface stress by the double stranded DNA formation from 10 nM to 100 nM), and the generation of surface stress by electrostatic force above 100 nM concentrations. As a result, we believe the microcantilevers are useful sensors for the detection of DNA hybridization, and these optimized surfaces, and assay conditions will contribute to the advanced clinical DNA detection based on the cantilever.

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ABSTRACT

Surface stabilization of piezoelectric thin-film driven microcantilever for label-free DNA detection

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The cantilevers have the mechanical structure, which is composed of the recognition layer on one side and the non-recognition layer on the other side. On account of this aspect, the cantilevers are facile to be influenced by non-specific binding of target or environmental biomolecules. In this thesis, to stabilize the surface and to minimize the nonspecific binding of target DNA, the microcantilevers were coated with perfluoro-silane. The optimal reaction condition is determined as dipping a cantilever in iso-octane at room temperature for 30 minutes. The surface stability under various conditions and the thickness of perfluoro-silane were confirmed by contact angle analyzer and XPS respectively. Based on the reliable surface, the resonant frequency shifts of the cantilever were measured as a function of the concentration between 10 nM and 5 μM. The resonant frequency shifts were increased from 10 nM to 100 nM, however they decreased up to 5 μM concentration. A tendency of signals according to the concentrations was explained as the release of

surface stress by the double stranded DNA formation from 10 nM to 100 nM), and the generation of surface stress by electrostatic force above 100 nM concentrations. As a result, we believe the microcantilevers are useful sensors for the detection of DNA hybridization, and these optimized surfaces, and assay conditions will contribute to the advanced clinical DNA detection based on the cantilever.

Key Words : **Microcantilever, Label-free DNA detection, Perfluoro-silane, Self-assembled monolayer, Surface stabilization**