

# Piezoelectric Based Detection of Cystic Fibrosis

## Part I: Review

Mark Austin, Andrew Baker, and Yousuf Mohammed  
Department of Electrical Engineering  
Northern Illinois University  
DeKalb, IL 60115 USA

**Abstract** – Improvements in medical technology over the past ten years has been growing remarkably. Deoxyribonucleic Acid (DNA) based biosensor technology is not absent from these vast improvements. This paper discusses an approach to detect the hybridization of the mutated cystic fibrosis gene. This is accomplished by immobilizing the single strand DNA that has been covalently bound to a biotin tag (biotinylated ssDNA) onto a biotin-streptavidin, gold coated quartz crystal probe. The hybridization of the cystic fibrosis transmembrane conductance regulator (CFTR) gene with its complement causes a change in mass in the quartz crystal microbalance which results in a measurable frequency change.

**Index Terms** – DNA Biosensor, Biosensors, Cystic Fibrosis, CF, Piezoelectric, Quartz Crystal Microbalance, Detection, Biotin, Streptavidin

### I. INTRODUCTION

A biosensor is a device that converts a biological response into a measurable signal. It consists of two elements; a sensing element and a transducer [1].

The sensing element is biological in nature and is often referred to as the substrate or analyte. A substrate is a molecule upon which an enzyme acts. The substrate in this paper is the single strand Deoxyribonucleic Acid (ssDNA) that is covalently bound to a biotin tag, which is referred to as a biotinylated ssDNA. This substrate is immobilized onto the biotin-streptavidin binding layers that are prepared on the gold surface of the piezoelectric probe. The biological elements are highly specific to that particular substrate. This prevents interferences from other substances [1]. In this case, the biological element is the biotinylated ssDNA that is immobilized on the piezoelectric probe.

The transducer converts the response of the sensing element into a measurable signal. A biosensor has a sensing element that has a specific response to the biological element desired to be measured. When the sensing element responds to the biological element, the transducer converts this response into a measurable signal for interpretation [1].

### II. DEOXYRIBONUCLEIC ACID

Deoxyribonucleic acid (DNA) is a nucleic acid that carries the genetic information used in the formation of living organisms. DNA is a double helix structure composed of two chains of nucleotides. Each nucleotide consists of a phosphate group, a deoxyribose sugar molecule, and one of four

different nitrogenous bases (adenine [A], guanine [G], cytosine [C] or thymine [T]) [2]. Each base is depicted in Fig. 1. Adenine and guanine are called purines and are similar in structure. Cytosine and thymine are called pyrimidines and are also similar in structure. Each base has a complementary base to which it pairs. Adenine pairs with thymine and guanine pairs with cytosine [2].

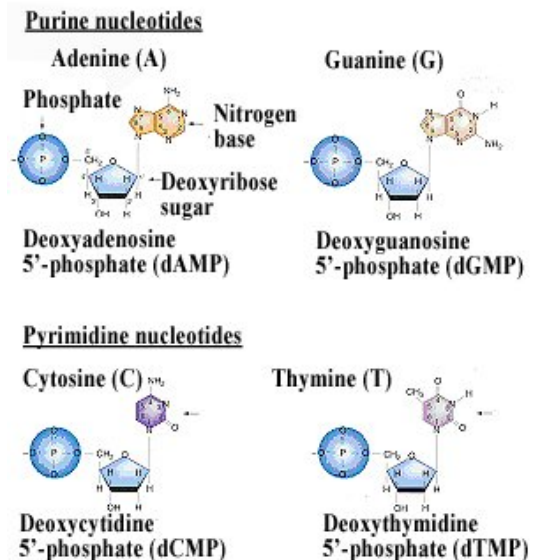


Fig. 1: Chemical structure of the four nucleotides. [2]

The backbone of the DNA is the phosphate-sugar complex that builds the strand. It is comprised of nucleotides covalently linked together by phosphodiester bonds. They are connected on the carbons (assigned numbers 1' through 5') on carbon 3' and 5'. One end of a single strand of DNA has a 5' phosphate group and the other a 3' OH group. The nucleotide chains are held together by weak hydrogen bonds between the bases [2]. A strand of four base-pairs is shown in Fig. 2.

A double strand of DNA has a certain melting temperature at which the strands separate. The temperature depends on the sequence length, the salt concentration, and the base composition. DNA with a high guanine and cytosine content has a higher melting point because G-C pairs have three hydrogen bonds compared to two hydrogen bonds in A-T pairs. The single strands of DNA will hybridize when the temperature is lowered back below the melting point. Optimal conditions for hybridization, for single strand DNA, are 25° C

below the melting temperature [2]. DNA can also be denatured using a dimethyl sulfoxide or formamide solution. The pH levels of these solutions cause the hydrogen bonds to separate [3]. In the current design, the ssDNA can be obtained from a biotechnology laboratory.

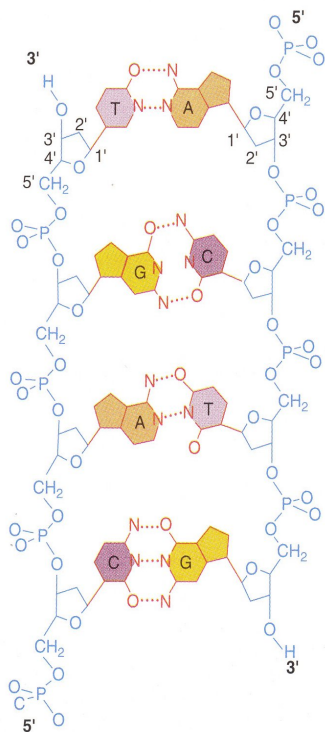


Fig. 2: A DNA double helix showing the sugar-phosphate backbone and base-pair rungs [2].

### III. CYSTIC FIBROSIS MUTATION

Cystic fibrosis (CF) is an autosomal recessive genetic disorder commonly found in Caucasians of northern European descent. It is caused by a mutation in the cystic fibrosis transmembrane conductance regulator gene (CFTR). A normal CFTR protein product is a chloride channel protein found in membranes of cells that line passageways of the lungs, liver, pancreas, intestines, reproductive tract, and skin [4]. The most common form of cystic fibrosis results from a deletion of three base pairs in the CFTR nucleotide sequence. This occurs in about 70% of cystic fibrosis patients. The mutation is caused by a loss of the amino acid phenylalanine located at position 508 in the protein located in q31.2 on the long arm of human chromosome seven (Fig. 3). The affected sequence is referred to as the  $\Delta F508$  CFTR sequence due to the deletion at that location (Fig. 4) [4]. Since it is a recessive gene, an individual must have two copies of the mutated CFTR gene to express the disease phenotype. Someone with one mutated copy of CFTR gene and one normal copy will be a carrier and will not express cystic fibrosis characteristics [4].

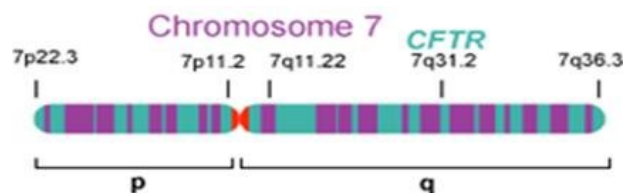


Fig. 3: Approximate location of CFTR based on chromosome 7 map from NCBI Entrez Map Viewer [4].

CFTR Sequence:					
Nucleotide	ATC	ATC	C	TTT	T GGT GTT
Amino Acid	Ile	Ile	Phe	Gly	Val
	506		508		510
			Deleted in $\Delta F508$		
$\Delta F508$ CFTR Sequence:					
Nucleotide	ATC	ATT	GGT	GTT	
Amino Acid	Ile	Ile	Gly	Val	
	506				

Fig. 4: The deletion of phenylalanine at location 508. The isoleucine at amino acid position 507 remains unchanged because both ATC and ATT code for isoleucine [4].

## IV. PIEZOELECTRIC EFFECT

### A. Quartz Crystals

Piezoelectric devices are very reliable means of converting mechanical vibrations into electrical energy. These transducers use a piece of polarized material with electrodes attached to two of its opposite faces. The piezoelectric transducers work on the principle of piezoelectric effect. Piezoelectric effect is the ability of certain crystals to generate electric charge in response to the applied mechanical stress. A Piezoelectric crystal produces an electric charge when a mechanical stress is applied and, conversely, a mechanical deformation is produced when an electric field is applied [5].

Piezoelectric transducers offer an attractive and near-universal mode of transducing the biorecognition event, but only if the detector mass that accompany the analyte binding are sufficiently large. In order to carry out a measurement, an external voltage is used to deform the quartz crystal plate so that there is a relative motion between the two parallel crystal surfaces at resonant frequency [6]. A change in frequency is the result of the mass that accompanies the analyte binding. This change in mass per unit area of the crystal is directly proportional to the change in frequency of the resonant crystal.

The DNA sequences of a few hundred base pairs have considerable molecular weights. This is described in further detail in the hybridization section. Due to the DNA hybridization at the biological sensing element of the sensor, there is change in mass and a change in the resonant frequency of the piezoelectric crystal [7]. The hybridization of the complimentary ssDNA on the biological sensing element is accomplished by immobilizing ssDNA on to quartz crystals and detecting the change in mass after the hybridization

process [1]. This results in a very large resolution and a very fast response time. The disadvantage exists with the possibility of intermediate hybrids resulting in an incorrect measurement.

### B. Theoretical Principles

The basic equations describing the relationship between the resonant frequency of an oscillating piezoelectric crystal and the mass deposited on the crystal surface is derived by the Sauerbrey equation [8]. The Sauerbrey equation is used in quartz crystal microbalance measurements. It expresses the correlation between the changes in oscillation frequency of a piezoelectric quartz crystal with the change of mass attached to the crystal [8]. This change in frequency is given by

$$\Delta F = -2.3 \times 10^6 F^2 \frac{\Delta M}{A} \quad (1)$$

where  $\Delta F$  is the change in frequency of the oscillating crystal measured in Hz,  $F$  is the frequency of the piezoelectric crystal measured in MHz,  $\Delta M$  is the mass of the deposited DNA due to hybridization measured in grams, and  $A$  is the area of electrode surface measured in  $\text{cm}^2$ .

The quartz crystal used is an AT-cut Quartz crystal. The AT-cut is singularly rotated about the y-axis as shown in Fig. 5. The top and bottom half of the crystal moves in opposite directions during oscillation [9].

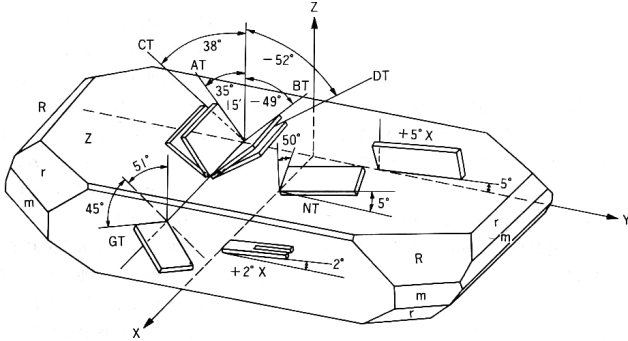


Fig. 5: Quartz crystal cutting angles [8].

The most widely used material for piezoelectric crystal detectors is AT-cut quartz or Alpha quartz crystals. AT-cut Quartz crystal is widely used because it can withstand high temperatures up to  $579^\circ\text{C}$  with no loss in piezoelectric properties. Quartz crystals such as AT-cut and BT-cut have zero temperature coefficients over a range of temperature and hence have good temperature-frequency characteristics. The Alpha cut or AT-cut is superior in temperature coefficient and sensitivity with change in mass compared to Beta cut or BT-cut piezoelectric crystals [11].

The oscillating frequency of an AT-cut Quartz crystal when vibrating in a thickness shear mode is given by

$$F = \frac{N}{t} \quad (2)$$

where  $F$  is the frequency of the crystal,  $N$  is the material constant (for AT-cut Quartz crystal it is 1.66MHz-mm), and  $t$  is the thickness of crystal plate.

For a change in frequency, the equation is given as

$$dF = d\left(\frac{N}{t}\right) = -N\left(\frac{dt}{t^2}\right) \quad (3)$$

For a finite amount of change, the above equation can be written as

$$\Delta F = -N\left(\frac{\Delta t}{t^2}\right) \quad (4)$$

Solving for  $N$  in (2) and substituting into (4), (5) can be given as

$$\frac{\Delta F}{F} = -\frac{\Delta t}{t} \quad (5)$$

Density is the mass per unit volume, so

$$D = \frac{M}{V} \quad (6)$$

where  $D$  is the density,  $M$  is the mass, and  $V$  is the volume.

Volume is given as, Volume = Area x Thickness or

$$V = A \times t \quad (7)$$

Therefore,

$$D = \frac{M}{(A \times t)} \quad (8)$$

The thickness  $t$  is given by

$$t = \frac{M}{(A \times D)} \quad (9)$$

Assuming constant density,

$$\Delta t = \frac{\Delta M}{(A \times D)} \quad (10)$$

Hence,

$$\frac{\Delta t}{t} = \frac{\Delta M}{M} \quad (11)$$

Thus from (5) one can say,

$$\frac{\Delta F}{F} = -\frac{\Delta M}{M} \quad (12)$$

Therefore,

$$\Delta F = \left( -\frac{\Delta M}{M} \right) F \quad (13)$$

Thus from (13), one can say that the oscillating frequency of the crystal changes linearly with the change in the mass of the crystal. This relationship is valid only if the changes in mass are small. For large mass changes this relationship fails as the density would change [8].

Therefore, if hybridization between the target DNA and the immobilized DNA on the piezoelectric crystal occurs, then there will be a change in mass in which the resonant frequency of the piezoelectric crystal changes and the mutation in the sample or target DNA can be detected [8].

## V. IMMOBILIZATION PROCESS

Over the past few years, different methods for immobilizing DNA on the surface of electrodes have been developed. Of all those methods used for sensing hybridization of DNA, piezoelectric quartz crystals are suitable for a direct and label free real time monitoring of affinity interaction between biomolecules. Quartz Crystal Microbalance (QCM) is a very sensitive device which uses the principle of mechanical resonance of the piezoelectric single crystalline quartz. The QCM is a very sensitive weigh device which is capable of measuring a change in mass as small as a fraction of a single layer of atoms [12].

Also, the use of self-assembled monolayers (SAMs) in various biomedical fields has grown rapidly. SAMs are used as an interface layer between the metal surface and the solution. The most commonly used metal for the formation of the monolayers is gold (Au) because it is reasonably inert. The uses of gold probes in detection systems or in biosensors have a number of advantages.

An 8MHz, AT-cut, Quartz crystal disc which has gold-plates on both faces will be used. The crystal is connected to an oscillation circuit and the frequency of the crystal is measured [12]. An example of the QCM setup is shown in Fig. 6.

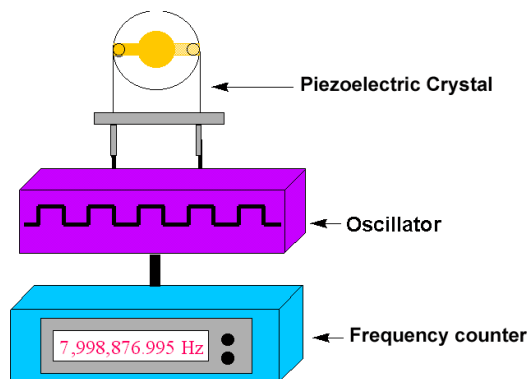


Fig. 6: QCM setup for detection of DNA hybridization [8].

To assure specificity and a reliable response, it is necessary to properly prepare the sensor surface. Therefore,

an appropriate chemical modification of the sensing surface is applied to the gold QCM surface. This procedure is ultrasonically cleaning the surface with a 20-min exposure to a Piranha solution ( $\text{H}_2\text{SO}_4:\text{H}_2\text{O}_2$ ) 3:1 [13].

The technique that is used for the detection of the mutated cystic fibrosis gene is a process where the mutated DNA sequence is Biotinylated. The gold surface on the piezoelectric crystal is coated with streptavidin. Avidin or Strept(avidin)-Gold conjugates can be used to detect the binding of Biotinylated molecules. Thus the target DNA needs to be Biotinylated so that they can interact with the avidin or streptavidin gold conjugates [14].

The biotin-strept(avidin) system has been used for many years in a variety of different applications. The interaction of avidin or streptavidin with biotin is characterized by a formation affinity constant of  $10^{15} \text{ L.mol}^{-1}$ . This affinity constant is much greater than the ligand-antibody interaction. Due to this high affinity between avidin or streptavidin and biotin, the complex formed is not disturbed by the changes in pH or during the multiple washings when it is immobilized [15]. Fig. 7 demonstrates the chemical structure of the biotin.

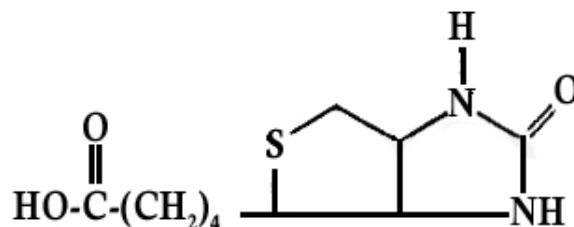


Fig. 7: Chemical structure of biotin [16].

Nucleic acid biotinylation can be obtained by several procedures. One of the well known methods is the transamination reaction of the cytosine residues with sodium bisulfite and a diiminoalkane like ethelenediamine. This reaction will work with ssDNA which can be prepared by heating a dsDNA at  $100^\circ\text{C}$  for about 3 minutes in the presence of both bisulfite and ethelenediamine. The product of this can be easily bionated by using an NHS-ester of biotin. By using this method one can obtain as much as 30-100 biotins per kilo base of DNA and can be controlled by adjusting the concentration of bisulfite and pH [15].

The gold surface of the piezoelectric crystal is coated with a layer of Biotinylated bovine serum albumin (BSA), Streptavidin and Biotinylated complimentary mutated Cystic Fibrosis ssDNA. This is shown below in Fig. 8.

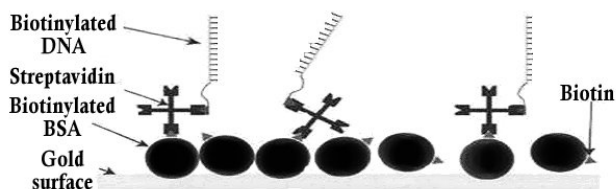


Fig. 8: A cartoon schematic of the protein binding layers for the surface preparation chemistry. Biotinylated bovine serum albumin (BSA) binds nonspecifically to the gold surface. Streptavidin, which has four biotin

binding sites, acts as a linker between the biotinylated BSA and the Biotinylated complimentary mutated CF ssDNA [17].

The ssDNA is Biotinylated by transamination reaction as discussed above. The bitinylation process covalently bonds the biotin to the ssDNA. The gold surface of the piezoelectric crystal is coated with Biotinylated BSA. Due to the high affinity between Streptavidin and biotin, it is very easy to make a layer of Streptavidin and biotin complex on the surface of the BSA layer [17]. Each Streptavidin has four equivalent binding sites for biotins. Streptavidin bonds with the biotin rapidly to form a complex which can tolerate a range of changes in pH and temperature conditions [17]. The Biotinylated ssDNA is introduced on the surface where the Streptavidin bonds with the Biotinylated ssDNA. This complex formed between Streptavidin and Biotinylated ssDNA is not disturbed or effected by the changes in temperature, pH, or due to washing that is done in order to test another sample of ssDNA.

Now, the piezoelectric crystal is immobilized with the mutated ssDNA and is ready for the hybridization process. The captured DNA sequence (shown in Table I) is the Biotinylated CF mutated DNA. The target DNA is the mutated ssDNA sample that is used for the hybridization.

TABLE I. DNA SEQUENCES FOR PROBE IMMOBILIZATION, HYBRIDIZATION, AND MUTATIONS [18], [19].

Capture DNA	5' TGC TGC TAT ATA TAT-biotin-3' Molecular Weight = 4636.97 daltons
Target DNA No CF gene	5' ATA TAT ATA GCA GCA GCA GCA GCA GCA GCA GAC GAC GAC GAC TCT C3' Molecular Weight = 14235.11 daltons
One-base mismatch	5' ATA TAT <u>AAA</u> GCA GCA GCA GCA GCA GCA GCA GAC GAC GAC GAC TCT C3' Molecular Weight = 14244.12 daltons
Three-base mismatch	5' ATA TAT <u>CCC</u> GCA GCA GCA GCA GCA GCA GCA GAC GAC GAC GAC TCT C3' Molecular Weight = 14172.05 daltons

## VI. HYBRIDIZATION

The molecular weight of the capture DNA that is immobilised on the probe is found out by using the molecular weights of adenine, guanine, cytosine and thymine. The calculated molecular weights are 312.2dalton for adenine, 328.2dalton for guanine, 288.2dalton for cytosine and 303.2dalton for thymine [19]. The equation is as follows,

$$\text{Molecular Weight of DNA} = (A * 312.2) + (G * 328.2) + (C * 288.2) + (T * 303.2) - 61.1 \quad (14)$$

The molecular weight of biotin is 244dalton [21]. The molecular weights are expressed in daltons or Da, 1 dalton is equal to 1.6605402E-24 grams.

Since the capture DNA sequence was biotinylated, which means tagged with biotin as described previously, and immobilized on the piezoelectric crystal, the total molecular weight is calculated and shown in Table I. By using the molecular weights in Table I, given above, the molecular weight of the ssDNA is calculated.

Since the capture sequence is biotinylated or tagged with biotin, the molecular weight of biotin is added to get the total molecular weight of the biotinylated sequence that is immobilized on our piezoelectric crystal.

For hybridization, the desired amount of the target ssDNA sample is added onto the piezoelectric crystal. It is carried out at a normal room temperature of 25°C or 77°F. Although the target DNA has no mutation, the DNA will still hybridize due the fact that there is only one base pair mismatch with the mutated ssDNA that is immobilized on the probe. For a single-base mismatch, the change in mass between the target DNA and the one-base mismatch is a total of 9.01 daltons. Using (13), this results in a frequency change of 5,060 Hz between the frequency of the target DNA and a one-base mismatch.

For a three-base mismatch, the change in mass between the target DNA and the one-base mismatch is a total of 63.06 daltons. Using (13), this results in a frequency change of 35,444 Hz. between the frequency of the target DNA and a three-base mismatch.

Therefore, the change in frequency of the piezoelectric crystal due to the change in mass after hybridization detects the presence of the mutation Cystic Fibrosis.

## VII. CONCLUSION

The process to create a piezoelectric based biosensor to detect the mutation of cystic fibrosis is demonstrated in this paper. It is shown that a complimentary ssDNA of the mutated cystic fibrosis gene can be immobilized to a quartz crystal microbalance probe using the biotin-streptavidin procedure. This probe proves to have a very large resolution and a very fast response time. Due to the electro-mechanical transduction process of the piezoelectric crystal, the biosensor is very reliable, small in size, and very cost effective. The piezoelectric material is also very stable, chemically inert, and has excellent mechanical properties, and has very good aging characteristics [11].

The bond between the two strands of ssDNA is just like any other bond of DNA, it can be broken with a simple washing of a piranha solution as mentioned earlier. However, this solution does not damage the strands, only the bond between the strands. Since the ssDNA is attached to the QCM probe via the biotin-streptavidin procedure, the binding is very robust and is not disturbed or effected by the changes in temperature, pH, or due to washing that is done in order to test another sample of ssDNA to detect the mutation. Therefore the bond between the ssDNA can be broken with the washing, while the biotinylated ssDNA remains attached to the QCM probe as shown in Fig. 8.

When hybridization occurs, the mass on the QCM will change, thus changing the resonant frequency of the quartz crystal. This proves to be a very effective method for the detection of cystic fibrosis and it is believed that this method

could be further implemented into a MEMS or 'Lab-on-a-Chip' technology.

#### REFERENCES

- [1] Eggins, Brian R.. Biosensors: An Introduction. USA: John Wiley & Sons, inc., 1996
- [2] Schmid, Silvan. "Immobilization of DNA and Protein to Polymerized SU-8 Photoresist Investigated with Microarray Assays." Swiss Federal Institute of Technology Zurich, 2003.
- [3] "DNA Denaturation." Molecular Station. 21 June 2009. <<http://www.molecularstation.com/dna/dna-denaturing/>>.
- [4] "CFTR: The Gene Associated with Cystic Fibrosis." (2003) <[http://www.ornl.gov/sci/techresources/Human\\_Genome/posters/chromosome/cftr.shtml](http://www.ornl.gov/sci/techresources/Human_Genome/posters/chromosome/cftr.shtml)>.
- [5] "Piezoelectric Transducers." Electronics Manufacturers. 2007. 9 Feb. 2009. <<http://www.electronics-manufacturers.com/products/sensors-transducers-detectors/piezoelectric-transducer/>>.
- [6] "Index of /electronique/piezo." 5 Apr. 2001. 9 Feb. 2009. <<http://www.aurelienr.com/electronique/piezo/piezo.pdf>>.
- [7] Junhui, Zhai, Cui Hong, and Yang Ruifu, "DNA Based Biosensors," Biotechnology Advances, 15.1 (1997): pp 43-58.
- [8] Kumar, Ashok. "Biosensors based on Piezoelectric Crystal Detectors: Theory and Applications." JOM-e: The Member Journal of TMS publication of The Minerals, Metals & Materials Society. October 2000. <<http://www.tms.org/pubs/journals/JOM/0010/Kumar/Kumar-0010.html>>.
- [9] "Quartz crystal microbalance." Wikipedia, The Free Encyclopedia. 26 Mar 2009, 22:13 UTC. 27 Mar 2009 <[http://en.wikipedia.org/w/index.php?title=Quartz\\_crystal\\_microbalance&oldid=279887744](http://en.wikipedia.org/w/index.php?title=Quartz_crystal_microbalance&oldid=279887744)>.
- [10] "What's Quartz Crystal Device." Quartz Crystal Industry Association of Japan. 1 Apr. 2009 <<http://www.qiaj.jp/pages/frame20/page01-e.html>>.
- [11] Hlavay, J., G.G. Guilbault. "Applications of Piezoelectric Crystal Detector in Analytical Chemistry." Analytical Chemistry. 49.13 (1977): 1890-1898.
- [12] Kupciunaite, J., A. Kausiate, A. Ramanavicius, and A. Ramanviciene. "Study of ssDNA immobilization and hybridization on gold substrate with quartz crystal microbalance." Department of Analytical and Environmental chemistry & Laboratory of Immunoanalysis and Nanotechnology. Vilnius University, Lithuania. 2006. <[http://images.katalogas.lt/maleidykla/Bio63/Bio\\_001\\_003.pdf](http://images.katalogas.lt/maleidykla/Bio63/Bio_001_003.pdf)>.
- [13] Steinem, Claudia, Andreas Janshoff, and M.A. Cooper. "Piezoelectric Sensors." Analytical and Bioanalytical Chemistry. 391.6 (July 2008): 2099-2100.
- [14] Hermanson, Greg.T. Bioconjugate Techniques. USA: Elsevier Science & Technology Books, April 2008.
- [15] Diamandis, E.P. and T.K. Christopoulos. "The Biotin-Strept(Avidin) System: Principles and Applications in Biotechnology." Clinical Chemistry 37 (May 1991): 625-636.
- [16] Jordan, Claire E., Anthony G. Frutos, Andrew J. Thiel, and Robert M. Corn. "Surface Plasmon Resonance Imaging Measurements of DNA Hybridization Adsorption and Streptavidin/DNA Multilayer Formation at Chemically Modified Gold Surfaces." Analytical Chemistry 69.24 (1997): 4939-4947.
- [17] Liu, Robin H. and Abraham P. Lee. Integrated Biochips for DNA Analysis. USA: Lands Bioscience and Springer Science, 2007.
- [18] Marin, Sergio, and Arben Merkoci. "Direct Electrochemical Stripping Detection of Cystic Fibrosis related DNA linked through Cadmium Sulfide Quantum dots." Nanotechnology. 20 (2009) 6pp.
- [19] "Molecular Weight Calculator." Encor Biotechnology Inc.. 2008. 1 Apr. 2009. <<http://www.encorbio.com/protocols/Nuc-MW.htm>>.
- [20] "DNA Calculations." MolGen. 3 Nov. 2008. 1 Apr. 2009. <<http://molgen.biol.rug.nl/molgen/files/DNAcalc.pdf>>.
- [21] "Binding of Biotin." CSEM. 1 Apr. 2009. <[http://www.csem.ch/detailed/b\\_471-EvaWIOS-LowMol.htm](http://www.csem.ch/detailed/b_471-EvaWIOS-LowMol.htm)>.

# Piezoelectric Based Detection of Cystic Fibrosis

## Part II: Sensor Design

Mark Austin, Andrew Baker, and Yousuf Mohammed  
Department of Electrical Engineering  
Northern Illinois University  
DeKalb, IL 60115 USA

**Abstract** – Improvements in Micro-Electro-Mechanical Systems (MEMS) technology has been growing remarkably over the past ten years. Deoxyribonucleic Acid (DNA) based biosensor technology is not absent from these vast improvements. This paper discusses an approach to implement a piezoelectric sensor to detect the hybridization of the mutated cystic fibrosis gene on a lab-on-a-chip. This is accomplished by using MEMS to construct the pumps and channels to transport the DNA to the piezoelectric sensor that detects the hybridization of the cystic fibrosis transmembrane conductance regulator (CFTR) gene with its complement. This causes a change in mass in the quartz crystal microbalance which results in a measurable frequency change.

**Index Terms** – DNA Biosensor, Biosensors, Cystic Fibrosis, CF, Piezoelectric, Quartz Crystal Microbalance, Detection, Biotin, Streptavidin, MEMS, Micro-Electro-Mechanical Systems

### I. INTRODUCTION

As described in *Piezoelectric Based Detection of Cystic Fibrosis Part I: Review* [1], this particular biosensor is used to detect the hybridization of the cystic fibrosis transmembrane conductance regulator (CFTR) gene with its complement. This is done so with a piezoelectric quartz crystal microbalance. When the complimentary cystic fibrosis (CF) single strand Deoxyribonucleic Acid (ssDNA) is introduced to the Biotinylated ssDNA that has been immobilized via the process described in [1], hybridization occurs. This occurrence causes a measurable frequency change in the quartz crystal microbalance.

This process can be implemented into a lab-on-a-chip (LOC) application. The miniaturization of the process using Micro-Electro-Mechanical Systems (MEMS) technology allows for the detection of CF without the time consuming process of sending samples to a lab for testing.

In this sensor design, four peristaltic polydimethylsiloxane (PDMS) micro-pumps are used to transfer the ssDNA to the piezoelectric transduction chamber.

### II. MEMS TECHNOLOGY

Miniaturization of electrical and mechanical systems has played a major role in technological development. The age of microelectronics began with the invention the transistor and integrated circuit (IC). Since then, the refinement and development of micro-fabrication techniques has provided the foundation for MEMS [2].

MEMS devices are made of a system of very small parts (on the micro scale) that are designed and fabricated as a unit

[3]. The concept of MEMS is currently being used in micro total-analysis systems ( $\mu$ TAS) and lab-on-a-chip (LOC) devices. The heart of a  $\mu$ TAS and LOC device is the analyte flow system made up of channels, pumps, valves, and mixers. The channels carry a sample that is propelled by pumps from the input of the device to the transducer. The micro-scale channels reduce the volume of samples and reagents used during analysis. Valves are used to direct the flow of the sample in the channels by only allowing it to travel in one direction. If two liquids need to be combined, a mixer is used. A simple mixer is made by splitting two channels into an array of smaller channels that are then merged into a single channel.

A  $\mu$ TAS and LOC device is capable of performing all the stages of chemical analysis such as sample-preparation, chemical reactions, analyte separation, analyte purification, analyte detection, and data analysis on a single chip [2]. What used to be done in a laboratory by specially trained technicians can now be done with a portable, battery powered device. Micro-fabrication allows for multiple  $\mu$ TAS to be fabricated on a single substrate such as silicon, glass, or a polymer [2].

### III. DESIGN OVERVIEW

The piezoelectric based detection of a cystic fibrosis DNA biosensor consists of an inlet and outlet port, piezoelectric transduction chamber, and micro channels connecting three pump chambers. The pump chamber consists of three valves that can be controlled by applying input power to heaters. A microcontroller is used to switch the input power. The sample under test, a single stranded DNA, is placed into the input port using a pipette. The sample ssDNA is pumped into the piezoelectric transduction chamber. The ssDNA hybridizes with the mutated ssDNA sequence which is immobilized on the piezoelectric crystal and a change in frequency occurs. This change in frequency can be measured using a frequency counter.

The left over sample ssDNA on the piezoelectric crystal surface is pumped through the valve to the waste chamber. The biosensor should be properly rinsed and washed before testing for other samples of cystic fibrosis. We use a piranha solution to rinse or wash the piezoelectric crystal surface. The device pumps the piranha solution through the channels, and then it is pumped to the piezoelectric crystal surface where it washes away the hybridized sample ssDNA; then it is pumped to the waste chamber.

## IV. FABRICATION

### A. MEMS

PDMS micro-pumps utilize a thermo pneumatic force to actuate the valves. Peristaltic type actuators are easily controlled by applying electric input power and can operate as dynamic valves. A single micro-pump consists of micro channels connecting three pump chambers with actuators. The actuators and chambers are fabricated with PDMS elastomer [4].

PDMS elastomer is very useful in fabrication of membrane type micro-pumps because of several advantages such as fast fabrication process, low cost, simple design, and flexibility. The PDMS elastomer can be used in fabrication of nano-liter or micro-liter level fluid control systems [4].

There are three different phases of sequences for the pump operation as shown in Fig. 1. When power is applied to the micro heater, the pressure in the pump chamber varies by ohmic heating of the air, causing the actuator to close. When voltage to the heater is decreased, the natural cooling of the air causes the actuator to open. The change in pressure in the pump chamber depends on the magnitude of the electric power applied to the heaters. In this design, one pump chamber is always closed; therefore, there is no backward fluid flow [4].

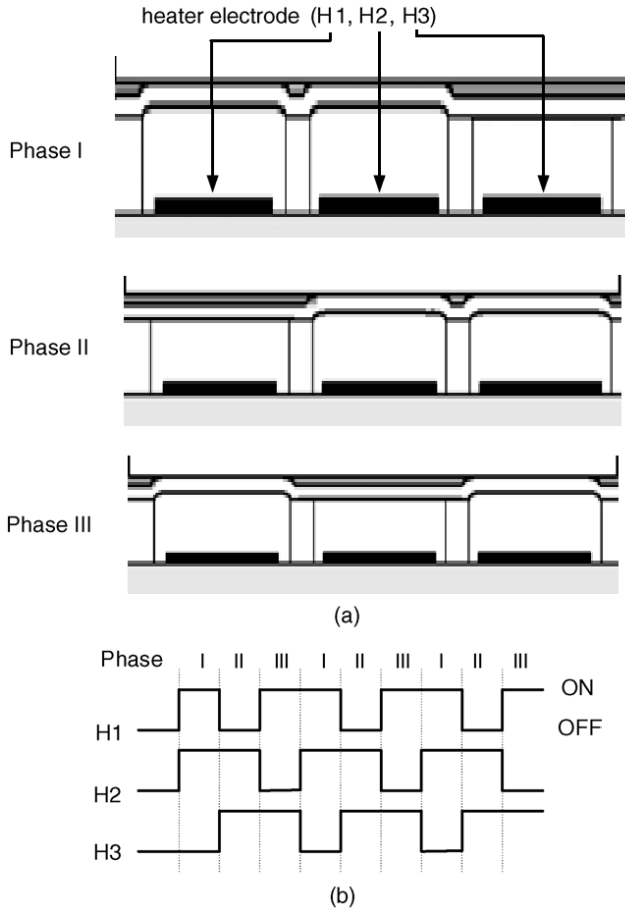


Fig. 1: Working principle of one PDMS micro-pump with three valves for a total of three different phases of actuation: (a) three phases of actuation; (b) applied input resulting in three different phases [4].

The micro-pump is fabricated by a PDMS elastomer by using sequential spin coating, a bonding process of PDMS, and soft curing techniques. The thickness of the PDMS elastomer on the surface of the silicon wafer during fabrication is controlled by a spin-coating process and a two step curing process that are an irreversible bonding process for PDMS to PDMS bonding [4]. Fig. 2 shows a top-down view and a cross-section view of the channels, pumps, piezoelectric chambers, electrode connection channels, and pump controllers.

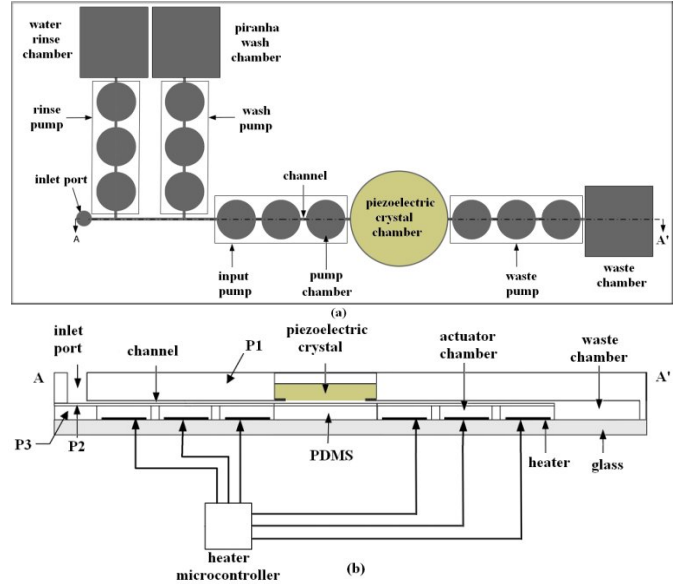


Fig.2: View of micropump (a) top down view; (b) cross sectional view and drive circuit; P1 is actuator chamber PDMS layer, P2 is actuator diaphragm PDMS layer, and P3 is micro-channel PDMS layer [4].

A 10:1 mixture of PDMS prepolymer and curing agent are thoroughly stirred and degassed in a vacuum chamber. This mixture is poured and spun at 500rpm for about 10 seconds and then softly cured at 65°C for about 15 minutes (Fig. 3 (a)) [4].

The cured PDMS layer is peeled off and three through holes are punched through it for an actuator chamber. The holes punched are 2.5mm in diameter. The actuator chamber layer is bonded with pyrex glass (#7740) and with a Cr/Au heater after oxygen plasma treatment for about 20 seconds (Fig. 3 (b)) [4].

The resistance of the heater is about 75Ω at room temperature. The actuator diaphragm layer is spun at 3000 rpm for 10 seconds and then cured at 65°C for about 15 minutes (Fig. 3 (c)) [4].

The PDMS prepolymer mixture is poured on the negative photo resist THB-151N, which is patterned for the pump chamber and the micro channel layer. Then the soft cured PDMS is peeled off from the pattern and inlet and outlet holes are punched for the fluidic connection [4].

The two peeled PDMS layers are irreversibly bonded for 10 minutes at 65°C. The flexible PDMS layer provides a continuous contact with the actuator diaphragm layer to prevent any bubbles from forming at the interface. The bonded pattern of PDMS is again peeled off and bonded with the bonded structure again for 10 minutes at 65°C after

oxygen plasma treatment for 20 seconds. The entire PDMS layers are cured again at 100°C for about an hour (Fig. 3 (e)) [4].

If the input voltage is 20V, the frequency for the maximum flow is 2Hz and the maximum flow rate for the pump is 0.36 $\mu$ L/s [4]. Fig. 4 shows the deflection of the actuators caused by the temperature rise within the pump chamber due to the rise in voltage applied to the heaters.

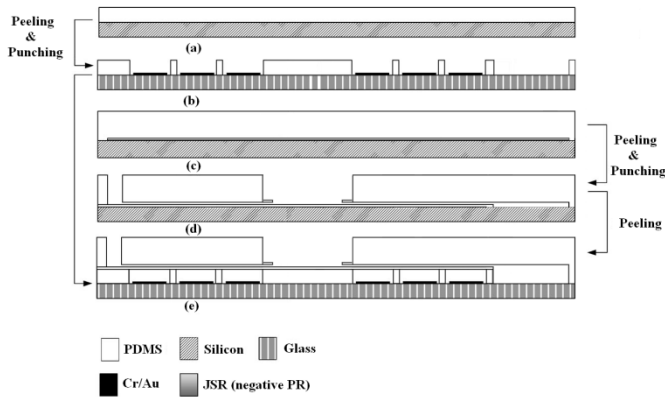


Fig. 3: Fabrication process of the PDMS micro pump: (a) curing of actuator chamber layer; (b) bonding of glass plate with actuator chamber layer and heater; (c) actuator diaphragm spin-coating and curing; (d) micro-channel casting (e) bonding of diaphragm and micro-channel layer [4].

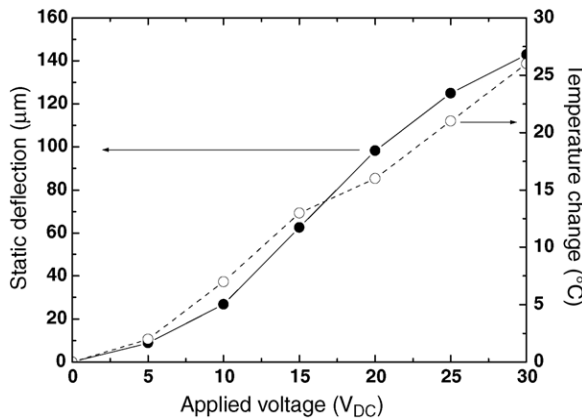


Fig. 4: Deflection of actuator and temperature change in pump chamber related to applied voltage to heater [4].

## B. Piezoelectric Sensor

The DNA sequences of a few hundred base pairs have considerable molecular weights. Due to the DNA hybridization at the biological sensing element of the sensor, there is change in mass and a change in the resonant frequency of the piezoelectric crystal [5]. The hybridization of the complimentary ssDNA on the biological sensing element is accomplished by immobilizing ssDNA onto a quartz crystal and detecting the change in mass after the hybridization process [6]. This results in a very large resolution and a very fast response time.

The fabrication of the actual sensor and immobilization of the DNA onto the sensor is performed as described in reference [1]. However, the required circuit for oscillation of the crystal is not discussed, as well as the circuit for the frequency counter. Therefore, the circuit shown in Fig. 4 is implemented. This circuit provides the quartz crystal with the ability to oscillate at a resonance frequency of 8M Hz.

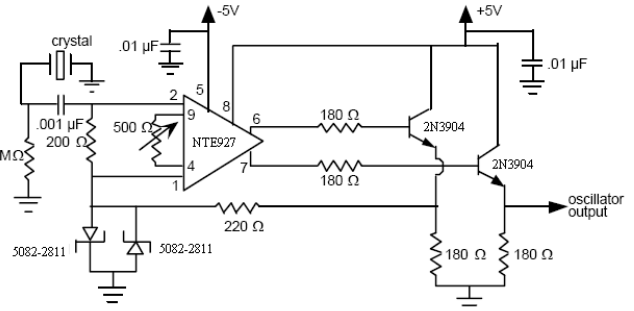


Fig. 4: Schematic for the oscillation circuitry for the piezoelectric crystal. [7]

The output from the oscillator is then fed into a frequency counter, shown in Fig. 5. The frequency counter displays the frequency of the crystal oscillation to within 1Hz. This will allow for the verification of the change in frequency as a result of the hybridization of the cystic fibrosis ssDNA.

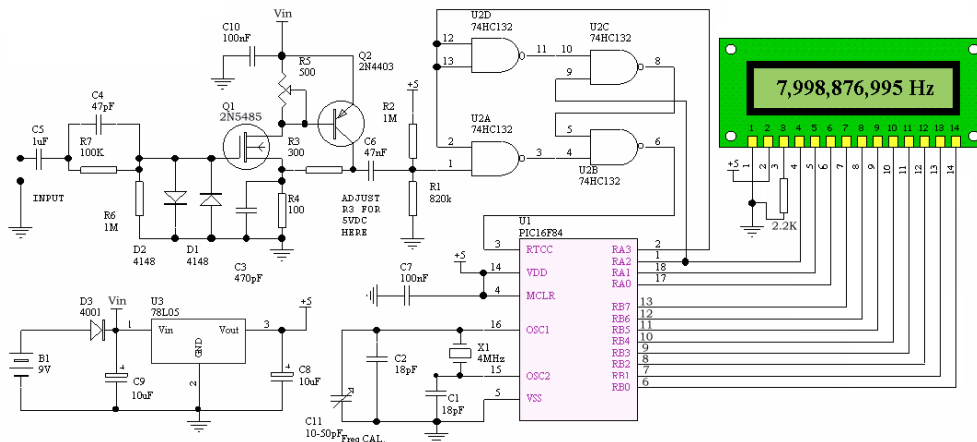


Fig. 5: Schematic for the frequency counter [8].

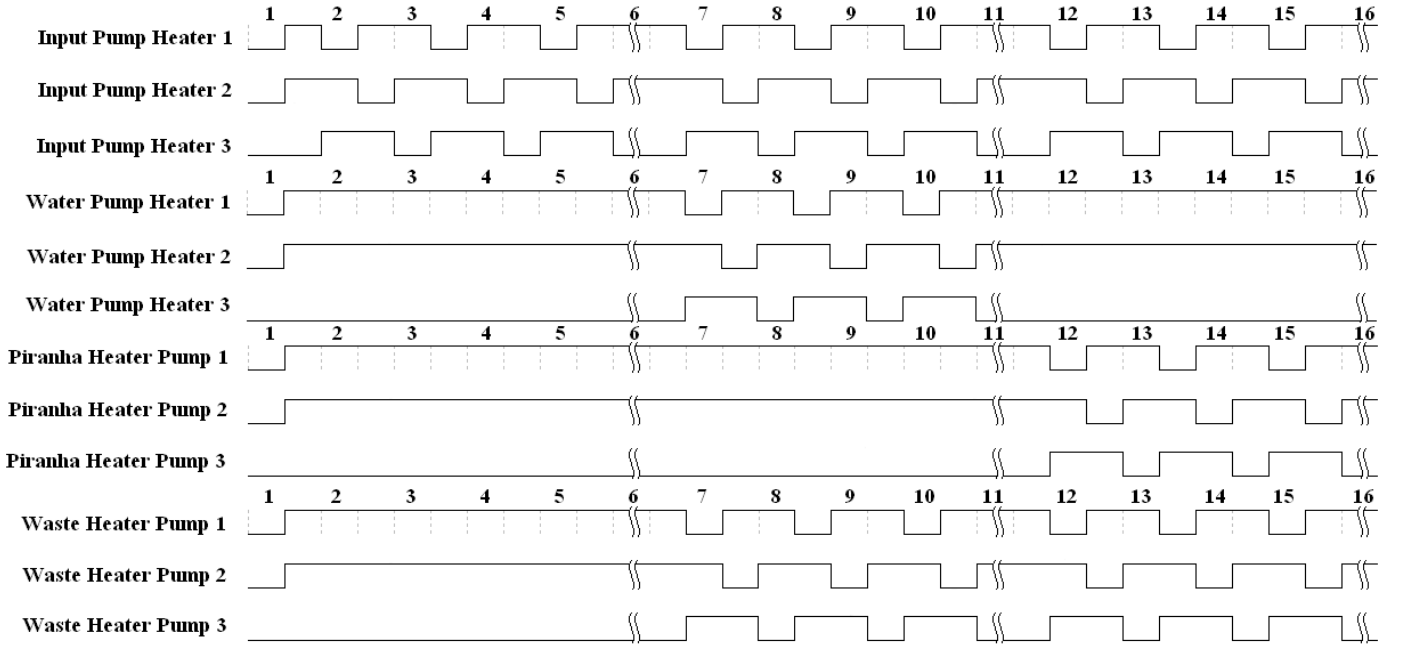


Fig. 6: Timing diagram of the pump heaters as described in Fig. 2 (a), showing the entire process from pumping sample into chamber to rinse then wash.

### C. Overall System

The complete system timing diagram is shown in Fig. 6; while Fig. 2 shows a top-down view and a cross-section view of the channels, pumps, piezoelectric chambers, and pump controllers. The input pump actuates and pumps the sample into the piezoelectric crystal chamber. Then the system waits 20 minutes for hybridization to occur. Then the water pump, input pump and waste pump actuate to flush out the remaining unhybridized sample. Then the frequency is measured with the frequency counter. After this, the piranha pump, input pump, and waste pump are actuated to break the DNA back into single strands and clean out the piezoelectric chamber. Then the device is ready for use again.

### V. DETECTION

Hybridization in the LOC will be detected using a piezoelectric sensor integrated with the microchannels. When the single strand DNA test sample is pumped into the piezoelectric chamber it will either hybridize or be pumped out. Hybridization will confirm the test sample contains the cystic fibrosis transmembrane conductance regulator gene  $\Delta F508$  mutation. To determine if hybridization occurs, the frequency of the piezoelectric crystal with the probe CF DNA attached will be measured using an oscillator and frequency counter. This frequency is represented by  $F$  in (1).

$$\Delta F = \left( -\frac{\Delta M}{M} \right) F \quad (1)$$

After the sample DNA is inserted into the channel and pumped into transducer chamber, the frequency of the piezoelectric crystal will be measured. If the frequency changes ( $\Delta F$ ), it can be concluded that there is a positive cystic fibrosis match. We can estimate the change in frequency by calculating the initial mass of the crystal and the

change in mass of the crystal and multiplying it by the measured frequency before the test sample was added. Using Table I, it can be shown that hybridization will cause a change in frequency of between  $3.056 * F$  and  $3.072 * F$ .

### VI. CONCLUSION

The process to create a lab-on-a-chip, piezoelectric based biosensor to detect the mutation of cystic fibrosis is demonstrated in this paper. In [1], it is shown that a complimentary ssDNA of the mutated cystic fibrosis gene can be immobilized to a quartz crystal microbalance probe using the biotin-streptavidin procedure. This probe will have a very large resolution and a very fast response time. Due to the electro-mechanical transduction process of the piezoelectric crystal, the biosensor is very reliable, small in size, and very cost effective. The piezoelectric material is also very stable, chemically inert, and has excellent mechanical properties, and has very good aging characteristics [9].

Using the PDMS micropump design, the device can be implemented into a MEMS system that results in a lab-on-chip design. The change in frequency can then be displayed on the LCD screen of the frequency counter informing the user that hybridization has occurred.

### REFERENCES

- [1] Austin, Mark, Andrew Baker, and Yousuf Mohammed. "Piezoelectric Based Detection of Cystic Fibrosis Part I: Review." Department of Electrical Engineering. Northern Illinois University, USA. 2009.
- [2] Geschke, O., H. Klank, and P. Telleman. Microsystem Engineering of Lab-on-a-Chip Devices. 2nd ed. Weinheim: Wiley-VCH, 2008.
- [3] Gardner, J.W., V. K. Varadan, O.O. Awadelkarim. Microsensors, MEMS, and Smart Devices. West Sussex: John Wiley & Sons, Ltd., 2001.
- [4] Jeong, O.K. Chan, et al. "Fabrication of a Peristaltic PDMS Micropump." Department of Electrical Engineering, Korea University, Republic of Korea. 23 Feb. 2005. 25 Apr. 2009.

<[http://wwwold.ajou.ac.kr/~mems/paper/S&A\\_2005-PDMS%20micropump.pdf](http://wwwold.ajou.ac.kr/~mems/paper/S&A_2005-PDMS%20micropump.pdf)>.

- [5] Junhui, Zhai, Cui Hong, and Yang Ruifu, "DNA Based Biosensors," Biotechnology Advances, 15.1 (1997): pp 43-58.
- [6] Eggins, Brian R.. Biosensors: An Introduction. USA: John Wiley & Sons, inc., 1996.
- [7] Ward, Michael. "Hardware and Circuit Components for a User-Constructed EQCM." Molecular Design Institute. New York University, USA.  
<<http://www.nyu.edu/fas/dept/chemistry/wardgroup/QCMcomponentslink.pdf>>.
- [8] "50 MHz Frequency Counter" Electronics-DIY.com. 2009. 23 Apr. 2009. <[http://electronics-diy.com/electronic\\_schematic.php?id=550](http://electronics-diy.com/electronic_schematic.php?id=550)>.
- [9] Hlavay, J., G.G. Guilbault. "Applications of Piezoelectric Crystal Detector in Analytical Chemistry." Analytical Chemistry. 49.13 (1977): 1890-1898.