# MEMS Based Cardiac Bio-enzyme Detection for the Acute Myocardial Syndrome Recognition

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***Abstract**:* - Early detection through enzymatic identification and confirmation is essential for diagnosis and prevention as in the case of Acute Myocardial Syndrome (AMS). Biochemical markers continue to be an important clinical tool for the enzymatic detection. The advent of Micro Electro Mechanical Systems (MEMS) devices can enable the use of various microstructures for the detection of enzymes. In this study, the concept of MEMS is applied for the detection of enzyme reaction, in which micro-cantilevers undergo changes in mechanical behavior that can be optically detected when enzyme molecules react on their surface, inducing geometric modifications. This paper presents the static behavior of micro-cantilevers under antigen-antibody reaction of rabbit skeletal muscle troponin C (TnC) and bee venom melittin (ME). The same troponin C is detected in the blood stream in humans a couple of hours form the acute myocardial infarction. The reported experimental results provide valuable information that will be useful in the development of MEMS sensors for enzymatic detection. The surface stress produced due to enzyme reactions results in the bending of cantilevers as similar to the influencing of laminar stress in the cantilevers. A possible design of such a system is provided in the paper.

***Key-Words**:* - Microsystems, Enzymatic detection, Acute Myocardial Syndrome, Troponin C, Bee venom melittin, Micro-cantilever, Surface stress, System design, MEMS.

## 1 Introduction

The identification of tiny volumes of biological material became a reality by mid ‘80s when the Polymerase Chain Reaction (PCR) technique discovered by Kary B. Mullis has opened a broad avenue to DNA identification and bio-material detection [1]. A significant effort over the past decade has been made to miniaturize, apart form the biological mass, the method and the equipment. Since the miniaturization of the equipment requires some automation, efforts towards the development of miniaturized equipment capable to handle the identification of biological mass have been made [2,3,4,5]. Efforts still are made as the diversity of the materials to be identified and the complexity and precision of the chemical reactions to be followed could be not fit in a single process flow [6,7]. This paper presents a miniature integrated miniature system capable to detect the biological reaction between specific heart enzymes and selected anti-enzyme. The system is intended to be used as a self-test device to enable the user to evaluate if it should be necessary to attend an emergency room or just to perform a routine test sometimes next

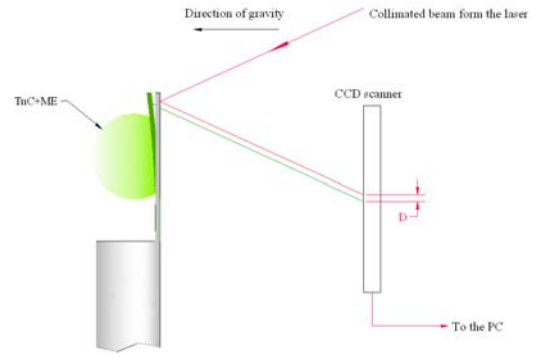
day. The system is based on the elastic deformation of a miniature beam under the biological reaction effect between minute amounts of enzyme and anti-enzyme.
Heart infarction is a very serious condition. However, over the past decade the tremendous progress made in the early detection and fast fix of the condition of the heart infarction have significantly contributed to the reduction of the morbidity of the illness [8]. On another side, the aging of baby-booming generation has been associated with the overloading of the medical system, situation which imposes some considerations. For an example, in North America over 33% of the emergency room requests are made by patients who claim a heart condition problem. However, out of those, only 12% have suffered condition that requires attentive medical attention and admission [9]. Situation may be similar other countries. However, a low cost and easy to use diagnostic tool available to the aging population who are risk subjects to heart illnesses. It is interesting to mention here the broad usage of the digital glucometers, systems that late ’80 were regarded as fancy and expensive equipment. Over the years they gained the popularity and found extensive usage. MEMS technology is an excellent candidate to yield such integrated systems. The major challenge is related to the requirements for the micro-system to handle liquids and measure deflection of elastic bodies while reducing the other associated phenomena induced by capillarity, electrostatic fields or gravity.

Preliminary work has been carried out by the authors who are now confident is stating that the enzyme-anti-enzyme reaction is detectable by means of the measurement of the elastic deformation pattern of a cantilever beam that hosts the two agents. Further challenges are in front of this research but however, an important step has been made toward the development of the entire system. The paper will present the results of the analytical and the experimental work as well as a planned configuration of the system, taking into consideration the above physical phenomena.

## 2 The Experiment

Preliminary work carried out by the authors has been focusing on the detection principle. However, two different enzymes were considered in the previous work [10]. The presented work is strongly supporting the previous results and drafts the architecture of the system design with more confidence. The biochemical reaction is carried out on a micro-cantilever beam while accurate tracking on the curvature of the cantilever is rigorously kept. Assuming a non-reactive solution, bending due to the extra weight is expected when a specific amount of solution is deposited on the micro-cantilever. After evaporation of the liquid phase, a smaller deflection in the direction of the gravity is expected. When tiny amounts of buffered enzyme and anti-enzyme are deposited on the cantilever, due to the bio-reaction, a different pattern is expected. Such pattern is proved and validated for multiple conditions.
The experiment has been carried out in the laboratory using an optical setup. The experimental setup is presented in figure 1. The features are scaled up (the cantilever beam) or down (the CCD scanner) to enable understanding of the experimental work. A collimated incident laser beam of 20 to 50 μm

diameter was centered with respect to the free end of the cantilever beam. The beam is reflected by the free end of the cantilever into a CCD scanner which output is written on a file in the in the PC. Measurements up to 100 times a second could be performed. However, this process is quite slow so for readings per second were set. The deflection of the cantilever could be measured through the displacement of the reflected beam on the cantilever, "D"[11].



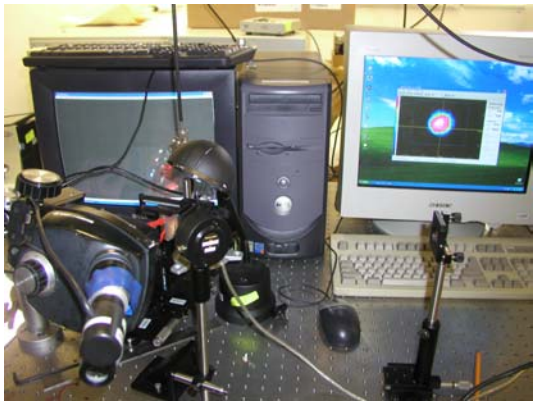



**Figure 1**. The experimental setup – schematics and detail

The TnC and the ME are mixed in equal proportion and various concentrations. The deposited amount of the beam is always same. For the experimental, polymer coated with aluminum beams of 2.5mm × 0.8mm were used such that larger amount of reactant were deposited (0.4µl). During the reaction between an enzyme and an anti-enzyme, the chains of enzyme molecules are opening and stretching such that some mechanical activity may be recorded on the cantilever [10]. Once the buffer solution evaporates, the remaining solid phase on the beam will arrange in a texture-like configuration such that this will create a permanent stress and therefore, a strain in the cantilever. This is observed as a displacement at the CCD scanner. Figure 2 illustrates the SEM of the TnC + ME after complete evaporation of the buffered solution. The long chains of the organic enzyme are observed being stretched and configured in a texture-like structure.

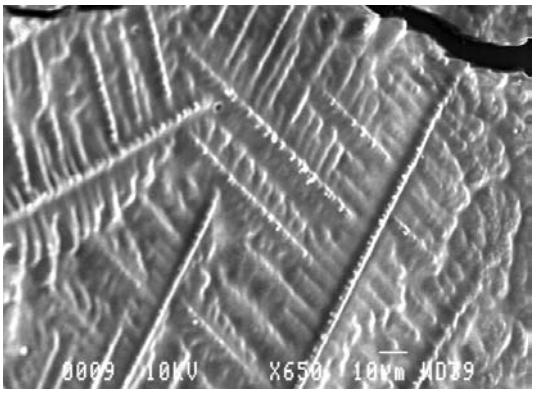


**Figure 2**. SEM of the TnC+ME after liquid phase evaporation

Measurement of the displacement "D" was performed under the circumstances for various concentrations of TnC. The pattern of the measurement regardless the concentration is always almost same and is illustrated in Figure 3. The displacement in µm at the CCD scanner in the vertical axis is given with respect to the time in seconds in the horizontal axis. The figure indicates that the loading with the organic fluid solution of the beam will create a deflection of about 10 µm that is translated at the CCD scanner in a negative (downwards) displacement of the laser spot by 400 µm. Further reactions will stretch more the beam due to re-organizing of the

enzyme molecules to the surface of the cantilever. The reactions count for 5 to 6 more μm of deflection at the cantilever

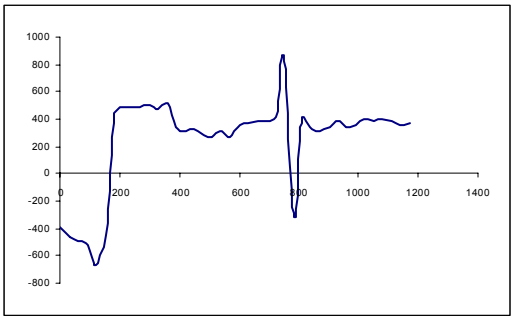



**Figure 3**. The record of the laser beam motion at the CCD scanner when 0.4 μl of TnC+ME is deposited

which occur over about one minute. The time duration is not quite relevant since the volumes used in the experiments were quite large. However, the displacement of 5 to 6 μm may be very easily detected in a microstructure. Further, the beam starts moving up by about 27 μm with respect to the lowest previous occupied position. This motion is slow at a rate of about 1 μm per second. This curving up is associated to the adhesion of the enzyme molecules on the surface of the cantilever which phenomenon produces a tension in the bottom surface of the beam. A long duration (about 500 seconds) almost steady condition occurs before an up, down and again up 25 μm jolt is seen at the CCD scanner, followed by a steady lever at almost same level as the previous steady level.

The relative steady pattern was compared to the one obtained by the deposition of the TnC + buffer solution or buffer solution. Significant differences are recorded in the pattern of the displacement and the displacement amplitude. The two patterns are presented in figures 4 and 5, respectively.

Given the above findings, the concept of a system of micro-systems to detect TcC is further proposed. However, of significant importance is to know the effect that the stress will have on a micro-cantilever beam.

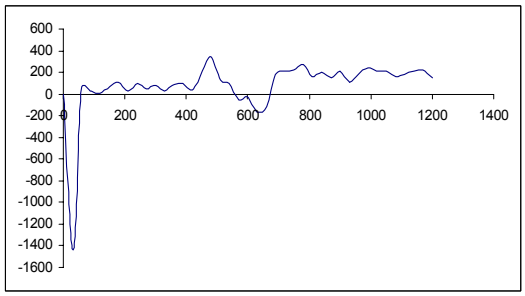



**Figure 4.** The deflection pattern recorded at the CCD scanner for TnC+buffer

Similar, for the buffer solution, the patter of the beam tip deflection is significantly different that the one recorded for the TnC+ME or just TnC+buffer solution. A displacement is recorded when the beam is loaded but there is no jolt or sudden displacement in the process.

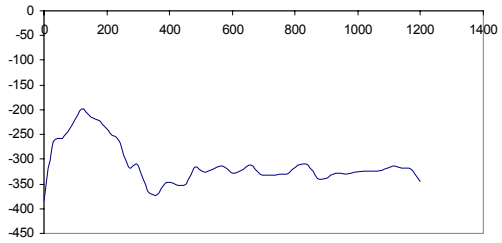



**Figure 5.** The deflection pattern recorded at the CCD scanner for buffer solution only

## 3 The Model

Although the deflection of the beam is observed, no reasonable explanation of the phenomena is available other that the structural re-configuration of the organic

molecules. However, since the enzyme molecules are attaching to the surface of the cantilever. The complex molecular motion could be considered as a stress induced in the beam. Figure 6 illustrates this condition. The wetting or non-wetting fluid on the surface of the beam may make a significant difference since the weight of the fluid is extremely small. As illustrated in Figure 6, a liquid that is wetting the surface may overcome the gravity and yield a upwards curvature although the gravity may indicate a different curvature.

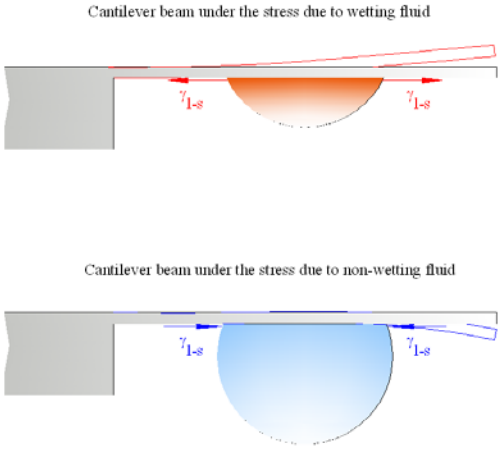



**Figure 6**. The effect of wetting and non-wetting of the fluid on the surface of a micro-cantilever beam.

The basic theory of beams could be used for the evaluation of the deflection in beams when subjected to stresses.
The strains in the micro-cantilever (ε) beam will be due to the initial pre-stress ($\varepsilon_0$), if any, the strain due to the load ($\varepsilon_y$) and the strain due to the capillary stress ($\varepsilon_c$).

$$\varepsilon = \varepsilon_0 + \varepsilon_y + \varepsilon_c \qquad (1)$$

The strains are unknown to the extent that the thermal built stresses will be assumed as pre-stress for the beam. The stress bending stress in the beam will be due to the pre-stress, load and non-symmetric capillarity:

$$\sigma_b = E\left(\varepsilon_0 + \frac{t}{2}\cdot\frac{d\theta}{dx} + \frac{\gamma_{l-s}}{l_d}\right) \qquad (2)$$

with E – Young modulus of the Si as the strongest material, t is the thickness of the beam, $\gamma_{l\text{-}s}$ is the capillarity coefficient for liquid-solid interface and $l_d$ is the distance on which the fluid is covering along the length of the micro-cantilever beam. The wetting could be controlled through surface coating or electrostatic charge.
The stress in the interface layer is assumed as being due to the capillarity forces. Since the same forces induce some bending in the micro-cantilever, the equilibrium forces in the axial direction of the beam could be written:

$$N = \int_{-b/2}^{b/2}\int_{0}^{t}\sigma_b dy dx + \int_{-l_d/2}^{l_d/2}\gamma_{l-s}dx = 0 \qquad (3)$$

or, if substitute (2) in (3),

$$N = \int_{-b/2}^{b/2}\int_{0}^{t}E\left(\varepsilon_0 + \frac{t}{2}\cdot\frac{d\theta}{dx} + \frac{\gamma_{l-s}}{l_d}\right)dy dx + \int_{-l_d/2}^{l_d/2}\gamma_{l-s}dx = 0$$

The maximum sensitivity will be encountered when $\frac{d\theta}{dx} = \max$ or:

$$\frac{d\theta}{dx} = -\frac{2\left[\gamma_{l-s}\cdot\left(\frac{t\cdot b + l_d^2}{l_d}\right)\right] + \varepsilon_0\cdot t\cdot b}{t^2\cdot b} \qquad (4)$$

Two distinct terms are defined by the sensitivity relationship, namely: the geometric and the fabrication term, which is constant for a specific design and which depends on the initial stress and the thickness of the cantilever beam, and the interface term that is direct proportional to the capillarity coefficient of the test solution on the solid surface of the cantilever beam.
If the geometric term of the curvature is assumed as constant, the deflection of the beam will increase with the increase of the term:

$$\frac{d\theta}{dx} = -2 \cdot \gamma_{l-s} \cdot \frac{t \cdot b + l_d^2}{t \cdot b \cdot l_d} \qquad (5)$$

The smaller is the thickness, higher is the sensitivity of the micro-cantilever. The contact stain of the fluid improves the sensitivity if the contact spot is either very small or very large.

When the fluid dries, the stress sets at a value due to the relaxation of the fluid on the structure on one side and due to the mass reduction on the beam, on another side.

One significant contribution towards reduced sensitivity would be the creation of fins on the fluid-deposition area of the cantilever beam such that the beam would bent more significant, meanwhile reducing the area of contact with the beam as illustrated in Figure 7.

## 4 The System

The above findings indicate towards a hybrid system that would contain various commercial technologies and some dedicated processes to build the highly sensitive cantilever beams. One of the significant challenges was the deposition of the reactants on one side of the micro-cantilever beam. A fluidic circuit in which the fluid is handled through capillary electrophoresis is also part of the circuit. Laser diode and optical positioning system are also necessary to be achieved in a commercial technology. A micro-controller could be easily implemented in a (Bi-)CMOS technology. The overall sensitivity of the device is expected to be very good although a large number of experiments should be run to validate the cross-sensitivity. Figure 7 illustrates the architecture of a system that could be accomplished by three to four different commercial technologies, a DRIE and a $XeF_2$ selective release of the mirror. The microfluidic device has to be filled in advance with buffer solution and the anti-enzyme for easy usage. Power could be attached as a long-lasting Li-ion battery.

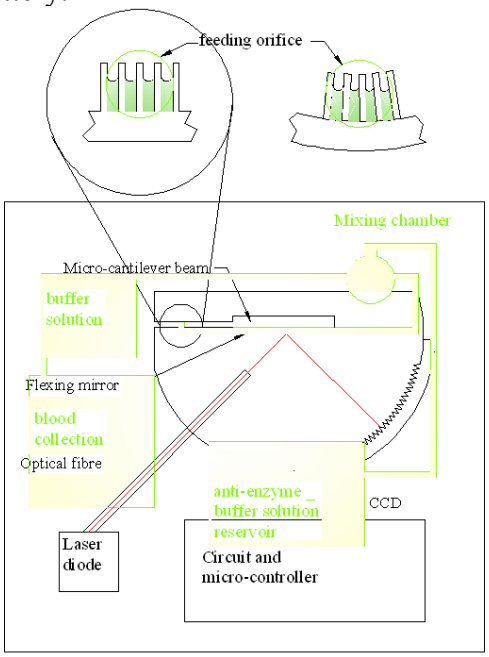



**Figure 7**. Possible architecture of the TnC detection system

## 5 Conclusions

The experimental results, the models and the available fabrication technologies point towards the feasibility of the realization of an integrated system capable to perform reliable readings for the TnC. The packaged kit may be assembled with a digital reader to provide objective readings of the troponin C level on the blood stream. A system if systems are proposed to perform the objective detection. A Si based chip that includes the laser diode, the CCD and the microcontroller is suggested. The optical devices could be realized separately and the assembly could be performed along with the micro-fluidic device. Further, a Deep Reactive Ion Etching is proposed to build the cantilever beam that could flex

in horizontal direction. After the selective etching is carried out, an isotropic gaseous phase etching is carried out to release the micro-cantilever mirror. The fluidic device is made from Pyrex 7740 glass such with anti-enzyme, buffer solution, and mixing chamber along with capability to move fluids in channels through electrophoresis. The device as conceived could be built in a 5000 × 5000 $\mu m^2$ chip surface. The device could be powered in a package by a long-life battery. The main advantage if such a device is that it could provide objective measurements of the TnC such that it could be preformed by a non-professional. Among challenges, one should enlist the fabrication of a mirror-like surface normal to the Si wafer, the cantilever release, the pre-loading of the micro-fluidic circuit with the anti-enzyme and the buffer solution, loading of the reactant on the cantilever without stiction.

## ACKNOWLEDGEMENTS

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