ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

INTEGRATION OF MICROFLUIDIC CHIP WITH A MICROCONTROL SYSTEM FOR STABILIZATION OF THE TEMPERATURE FOR DIAGNOSIS OF TUBERCULOSIS BY LOOP MEDIATED ISOTHERMAL AMPLIFICATION

M.Sc. THESIS

Zeynep Burcu ÇAVDAR

Department of Nanoengineering and Nanoscience

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Zeynep Burcu ÇAVDAR
(513141024)

Department of Nanoengineering and Nanoscience

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Thesis Advisor: Prof. Dr. Levent TRABZON

DECEMBER 2017

Zeynep Burcu ÇAVDAR a M.Sc. student of İTU Graduate School of Science Engineering and Technology student ID 513141024., successfully defended the thesis/dissertation entitled "Integration Of A Microcontrol System With Microfluidic Chip For Stabilization Of The Temperature On The Microheater For Diagnosis Of Tuberculosis Infection By Loop Mediated Isothermal Amplification", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

Thesis Advisor :	**Prof. Dr. Levent TRABZON** İstanbul Technical University	.....
Jury Members :	**Doç. Dr. Mustafa BAKKAL** Istanbul Technical University	.....
	Prof. Dr. Haluk KÜÇÜK Marmara University	.....

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To my family and friends,

FOREWORD

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Zeynep Burcu ÇAVDAR
(Chemical Engineer)

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ABBREVIATIONS

Al	**:** Aluminium
LAMP	**:** Loop Mediated Isothermal Amplification
MEMS	**:** Micro Electronical Mechanical Systems
OP-AMP	**:** Operational Amplifier
PCB	**:** Printed Circuit Board
PCR	**:** Polymer Chain Reaction
PDMS	**:** Polydimethylsiloxane
TB	**:** Tuberculosis
TB-LAMP	**:** Tuberculosis detection kit via Loop mediated isothermal amplification
WHO	**:** World Health Organisation

SYMBOLS

C	**:** Capacitance
d_s	**:** Thickness
J^e	**:** Internal current density
k	**:** Thermal conductivity
n	**:** Normal vector
Q_j	**:** Current source
R	**:** Resistance
T	**:** Temperature
V	**:** Voltage
α	**:** The temperature coefficient of resistivity (TCR)
ρ_0	**:** The resistivity at the reference temperature
σ	**:** Temperature

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INTEGRATION OF A MICROCONTROL SYSTEM WITH MICROFLUIDIC CHIP FOR STABILIZATION OF THE TEMPERATURE ON THE MICROHEATER FOR DIAGNOSIS OF TUBERCULOSIS INFECTION BY LOOP MEDIATED ISOTHERMAL AMPLIFICATION

SUMMARY

Tuberculosis is the one of the most worldwide studied disease which has high mortality rate from ancient time to modern days. According to World Health Organization (WHO) statistics in 2015, 10.4 million of people were infected with tuberculosis and 2 million of them were died. *Mycobacterium tuberculosis* is an acid-fast, gram positive pathogen bacterium with rod shape and 2-4 μ length. Although it pertains to *Mycobacterium* specie which could be found in soil and water reservoirs a lot, *Mycobacterium tuberculosis* effects most of the parts in human body, especially it infects lungs by settling on to alveoli walls. This bacterium has a tendency to spread through air: therefore, patients can throw it out from their body by the way of phlegm and small aerosols. This causes to infect another person by flying on the air. When this bacterium, which is light sensitive, is exposed to sun light, it dies in 2 hours however, it can resist in phlegm for 20-30 hours. Also, reproduction time is 12-24 hours but lives in 2-8 months at dried conditions.

Although tuberculosis has a history from ancient times, there wasn't a certain cure until 1950's. In 1950's with the help of antibiotic usage became common, tuberculosis was cured in success. Even these days antibiotics are still used to treat tuberculosis. Standard treatment period is 6 months as antibiotics of Izoniazid, Rifampisin, Streptomisin, Etambutol are used in first 2 months. Most important factors to help treatment successful are fast and accurate diagnosis and prevention of resistivity during treatment period. Especially resistivity of multiple drugs are very common nowadays. Each year 500.000 new resistivity of multiple drug cases of tuberculosis are reported however 30.000 of the could be diagnosed.

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In the following researchs, new amplifications were invented and denaturization step was eliminated. Loop Mediated Isothermal Amplification (LAMP) method is one example of that. This method is applied to detect the evolution of pathogens, especially used in food industry pathogens. Advantages compared to PCR method are less primer needed, faster and specific results obtained, no need for denaturization process and no need for heater or water bath in laboratory. Rise of DNA amount, follow up of increasing turbidity in solution, absorbance data by color change due to realease of fluorescence material binds to DNA and follow up of magnesium concentration in environment by staining can be controlled. Because of that, the system fast and high sensitive data is obtained without expensive and big equipments,. Results can be analysed by a simple optic system or by naked eye. LAMP technoque provided results with 96.8% susceptibility and 100% individuality.

Lab on a Chip technology is commonly used in IVD tests in recent studies. Especially polymeric and capable to integrate most of the systems micro scale systems which is called as microfluidic systems are important to make a few tests on it instead of in a laboratory. LAMP reactions can be seen as used in microfluidic chips for pathogen applications in literature studies. Also, Tuberculosis bacilli isolation form phelgm, isolation of DNA from bacilli, replication of the isolated DNA and point mutations can be detected by several studies as a matter of fact some of them are already commercilized.

This Master thesis was supported by TUBITAK with project code of 115R002. Aim of this project is to reproduce isolated DNA from patient's phelgm by LAMP method with stabilized temperature by microcontroller on microfluidic chip and in case od success, this prepared microchip is aimed to be commercilized. This project has 5 main progresses: design, production and characterization of microfluidic chip with heating element and reaction chamber where reaction is occured, circuit of microcontroller design to control temperature, characterization and integration, preparation of biological mixture to fill reaction reservoir and optimization, filling prepared biological solution to the reaction reservoir in prototype and optimization of prototype according to data obtained and determination of shelf life of the prototype. In this thesis not only for microfluidic chip but also for microcontroller: production, design, modelling and characterization were done also analysis of the data obtained was interpreted.

LAMP YÖNTEMİYLE TÜBERKÜLOZ TEŞHİSİNDE KULLANILMAK ÜZERE ÜRETİLEN MİKROAKIŞKAN ÇİPİN SICAKLIK KONTROLÜ İÇİN MİKROKONTROL DEVRESİYLE ENTEGRASYONU

ÖZET

Tüberküloz, en eski çağlardan günümüze dek ölüm oranı en yüksek hastalıklardan biri olarak birçok ülkede en yoğun çalışılan hastalıklardan biri olmuştur. Dünya Sağlık Örgütü (WHO) verilerine göre 2015 yılında 10.4 milyon kişi bu hastalığa yakalanmış ve 2 milyon Tüberküloz hastası hayatını kaybetmiştir. Özellikle gelişmekte olan ülkelerde daha yüksek oranda görülmekte olan bu hastalığa neden olan Mycobacterium tuberculosis; gram-pozitif, çubuk şeklinde, aside dirençli, 2-4 μm uzunluğunda patojen bir bakteri türüdür. Dahil olduğu Mycobacterium ailesi toprak ve suda bol miktarda bulunsa da, Mycobacterium tuberculosis insan vücudunda birçok bölgeyi etkilemekte, özellikle akciğer alvoel duvarlarına yerleşerek akciğerleri enfekte etmektedir. Havada yayılma özelliğine sahip bu bakteri, hasta tarafından balgam ve küçük aerosol damlacıklar içerisinde vücut dışına atılabilir ve havada uçuşarak başka insanları enfekte edebilir. Isıya duyarlı olan bu bakteri, güneşe doğrudan maruz kaldığında 2 saat içerisinde ölürken, balgam içerisinde 20-30 saat dayanıklı kalabilir. Ayrıca, çoğalma süresi 12-24 saat olup kuru ortamda 2-8 ay canlı kalabilir.

Tüberkülozun tarihçesi çok eskilere dayansa da 1950'lilere kadar kesin bir tedavisi bulunmamaktaydı.1950'lerde antibiyotik kullanımının yaygınlaşmasıyla birlikte Tüberküloz hastalığının tedavisinde de başarılı olduğu görülmüş ve günümüzde en etkin tedavi yöntemi olarak çeşitli antibiyotikler kullanılmaya devam edilmektedir. Standart tedavi süresi 6 ay olup ilk 2 ay İzoniazid, Rifampisin, Streptomisin, Etambutol gibi antibiyotikler kullanılır, kalan 4 ayda ise Rifampisin ve Izoniazid kullanımına devam edilir. Hastalığın tedavisinin başarılı olmasında rol alan en önemli iki etkenden biri hastalığın hızlı ve doğru biçimde teşhis edilmesi, diğeri de tedavi sırasında kazanılmış direnç gelişimini önlemektir. Her ne kadar etkili bir tedavi uygulansa da çeşitli etkenler bakterinin ilaca karşı direnç geliştirmesine neden olmaktadır. Özellikle son yıllarda çoklu ilaç direncine sahip bakteri sayısı giderek artmaktadır. Her yıl yaklaşık olarak 500,000 yeni çok ilaca dirençli (ÇİD) tüberküloz (TB) bildirilmesine rağmen bunların yalnızca 30,000 kadarı tanımlanıp, rapor edilebilmektedir.

Tüberküloz hastalığının tedavisindeki en büyük etmenlerden biri hastalığın hızlı teşhis edilmesidir. Mevcut tanı sistemi akciğer grafisi ve tüberküloz deri testi olup, laboratuvarda çeşitli boyama ve kültür testlerine tabi tutulmaktır. Güçlü bir peptidoglycan yapısına sahip olması nedeniyle boyama testleri biraz zayıf kalmaktadır. Bu testler, hastalığı teşhisinde yardımcı olsa da ilaca dirençli bakterilerin varlığı tedaviyi zorlaştırmaktadır. Ayrıca, boyama testleri birkaç gün içerisinde sonuç verse de kültür testleri birkaç haftayı bulabilmektedir. Bu süre zarfında hastalığın hem

bulaşması hem de ilerlemesi riskleri bulunmaktadır. Bunun önüne geçilmesi için de hem hızlı hem de güvenilir sonuç veren yeni tekniklere ihtiyaç duyulmaktadır.

"In vitro diagnosis" yani "Hücre dışı teşhis" testleri günümüzde hem laboratuvarlarda hem sanayide hem de evlerde, çeşitli hastalıkların, enfeksiyonların veya durumların hızlı ve hassas bir şekilde teşhis edilmesinde etkin rol oynamaktadır. Bugün tüm dünyadaki IVD sektörünün %30'unu hasta başı testler oluşturmaktadır. ABD'de pazardaki payı >13 milyar dolardır ve 20-25 yıl önce 7-8 test iken şimdi sayıları yüzleri, bulmaktadır. Tüberkülozun teşhisinde, hastadan alınan örneklerin kalitesi ve miktarı çok büyük sorunlara neden olabilmektedir. Özellikle balgamdan bakteri izolasyonu ve izole edilen bakteriden DNA izolasyonu sırasında yaşanan kayıplar ile balgamda Tüberküloz basili ile birlikte başka bakterilerden ve balgamın kendisinden de gelen DNA kontaminasyonu nedeniyle elde edilen DNA için istenilen spesifiklikte elde edilmeyebilir. 1980'lerde ortaya çıkan, Polimerik Zincir Reaksiyonu (PCR) olarak adlandırılan yöntemde yüksek sıcaklıkta DNA'nın denature edilmesi ve sıcaklığın düşürülerek DNA polimeraz adı verilen ve DNA replikasyonunda görevli bir enzim tarafından, altı ya da daha fazla primer yani küçük RNA parçaları kullanılarak DNA replikasyonun gerçekleşmesi ve tekrar yüksek sıcaklıkta DNA denaturasyonunu izleyen bir sıcaklık döngüsü izlenmektedir. Eldeki örnekten izole edilmiş çok düşük miktardaki DNA'nın bu yöntemle çoğaltılması istenilen spesifiklik ve miktarda DNA üretimi sağlanmaktadır. İleriki zamanlarda yeni amplifikasyon yöntemleri ortaya çıkmış ve denaturasyon adımını kaldırılmasını sağlayan optimizasyonlar yapılmıştır. "Loop Mediated Isothermal Amplification (LAMP)" yöntemi de bunlardan biridir. Literatürde "İlmiğe Dayalı İzotermal Amplifikasyon" olarak çevrilmiş birkaç makale mevcut olsa da "Loop Mediated Isothermal Amplification (LAMP)" yönteminin dilimizde tam karşılığı bulunmamaktadır. Özellikle, yiyecek sanayisindeki patojenlerin tespitinde yaygın bir kullanım alanı bulmaya başlayan bu yöntemin PCR'a göre avantajları, daha az primer kullanımı gerektirmesi, daha hızlı ve spesifik sonuç alınması, denaturasyon işlemine ihtiyaç duymaması ve sabit sıcaklıkta çalışılması gerekliliğinden dolayı laboratuvar içerisindeki herhangi bir ısıtıcı ya da su banyosunda çalışabiliyor olması şeklinde sıralanabilir. DNA miktarındaki artış, çözeltideki bulanıklığın artışının takibi ve DNA'ya bağlanan fluresans maddenin salınması sonucu renk değişiminin absorbans değerlerininölçülmesi veya ortamdaki magnezyum konssantrasyonunun takibini sağlayan boyaların kullanılması ile takip edilebilmektedir. Bu nedenle, pahalı ve büyük ekipmanlara ihtiyaç duyulmadan hızlı ve yüksek hassasiyetle sonuç alınabilmektedir. Sonuçlar, basit bir optik sistemle veya çıplak gözle değerlendirilebilmektedir. LAMP yönteminde %96.8 duyarlılık ve %100 özgüllükte sonuçlar elde edilebilmektedir.

IVD testlerine bakıldığında Lab on-a-chip yani minyatür laboratuvarların kullanımının son yıllarda yaygınlaştığı görülmektedir. Özellikle mikroakışkan sistemler olarak adlandırılan ve içerisinde bir laboratuvarda yapılan bir çok testin uygulanabildiği polimerik ve başka sistemlere entegre edilebilen mikro ölçekteki sistemler birçok alanda kendine yer bulmaktadır. Literatüre bakıldığında, LAMP reaksiyonunun mikroakışkan çipler üzerinde farklı patojenler için uygulamaları görülmektedir. Ayrıca, Tüberküloz basilinin balgamdan izolasyonu, basilden DNA izolasyonu ve izole edilen DNA'nın replikasyonu ve nokta mutasyon tespiti için farklı farklı çalışmalar olduğu hatta çalışmalardan bir kısmının da ticari ürün olarak piyasaya sürüldüğü görülmektedir.

Bu tez, TÜBİTAK tarafından 115R002 nolu proje ile desteklenmiştir. Projenin amacı, hastadan alınan balgamdan izole edilen DNA'nın sıcaklık kontrolü entegre bir mikrokontrol devresiyle sağlanan mikroakışkan çipte sabit sıcaklıkta LAMP reaksiyonuyla çoğaltılması ve başarılı olunması halinde hazırlanan mikroakışkan çipin ticarileştirilmesidir. Belirtilen proje beş ana aşamadan oluşmaktadır: Isıtıcı ve sıcaklık sensöründen oluşan bir ısıtma elemanı ile reaksiyonun gerçekleşeceği reaksiyon haznesini içeren bir mikroakışkan çip tasarımı, üretimi ve karakterizasyonu, sıcaklık kontrolünü sağlamak için mikroakışkan çipe entegre edilecek bir mikrokontrol devresini tasarlanması, karakterizasyonu ve entegrasyonu, reaksiyon haznesine yüklenecek biyolojik madde karışımının hazırlanması ve optimizasyonu, üretilen prototipe hazırlanan biyolojik karışımın yüklenmesi ve alınan sonuçlara göre prototipin optimizasyonu ve prototipin raf ömrünün belirlenmesi. Bu tezde, hem mikroakışkan çipin hem de mikrokontrol devresinin tasarım, modelleme, üretim ve karakterizasyon aşamaları incelenmiş, karakterizasyon işlemlerinden alınan sonuçlar yorumlanmıştır.

Isıtma elemanı, Joule kanununa uygun şekilde tasarlanmış ve hızlı ısıtma için Alüminyum ana malzeme olarak seçilmiştir. Isıtma elemanına ait elektrik-sıcaklık bağlantıları 'COMSOL Multiphysics' adı verilen 'Sonlu Elemanlar Methodu' tabanlı bir simülasyon programı ile modellenmiş ve alınan sonuçlara göre tasarım optimize edilmiştir. Isıtma elemanı için simülasyon sonuçları ve elektrik bağlantıları yapılırken karşılaşılan sorunlar göz önüne alınarak Al kaplama kalınlığı 300 nm olarak optimize edilmiş ve reaksiyon haznesi PDMS adı verilen oksijen geçirgenliği yüksek polimerik bir malzeme kullanılarak hazırlanmıştır. Ayrıca, ısıtıcı elemanı üzerine ısı dağılımının daha homojen hale getirilmesi amacıyla SU-8 adı verilen polimerik bir malzeme ile 15 μm kaplama yapılmıştır.

Isıtma elemanının elektrik bağlantıları, elektronikte özellikle mikroçip paketleme alanında geniş bir uygulama alanına sahip olan 'Wire Bonder' adı verilen ekipman kullanılarak yapılmıştır. Elektrik bağlantısı için 33 μm kalınlığında Al tel kullanılarak kama bağlama yöntemi uygulanmıştır. Telin kalınlığının çok düşük olması ve çoklu kullanımda zarar görmesinin engellenmesi amacıyla elektrik bağlantılarının yapıldığı bölgelerein üzeri epoksiyle kaplanmıştır. Üretimin her aşamasında ve elektrik bağlantıları yapımından sonra ısıtma elemanına ait voltaj-direnç ve sıcaklık- direnç ölçümleri yapılmış ve ölçüm sonuçlarına tezde yer verilmiştir.

LAMP reaksiyonunda yüksek hassasiyette çalışılması için en önemli parametre olan sıcaklığın belirli bir aralıkta tutulması amacıyla bir mikrokontrol sistemi hazırlanmış ve elektrik bağlantıları tamamlanmış olan mikroakışkan çipe entegre edilmiştir. Mikrokontrol devresinin sisteme entegrasyon çalışmalarında Arduino Uno adı verilen Atmega328 model mikrodenetleyici içeren hazır ticari bir kart kullanılmaktadır. Mikrokontrol sistemi ayrıca INA125 adlı sensördeki direnç değişimini algılayarak sonucu mikrodenetleyiciye besleyen bir voltaj çoğaltıcısı ve ısıtıcının güç kaynağını kontrol eden bir adet transistör içermektedir. Arduino Uno hem kullanımı kolay bir arayüz sunması hem de alınan sonuçları takip etmekte ve işlemekte sunduğu kolaylıklar göz önünde bulundurularak seçilmiştir. Entegrasyon işlemleri tamamlandığında hassasiyetin arttırılması için Atmega 32 model mikrodenetleyiciye geçiş yapılması planlanmıştır.

Ina125 adlı voltaj çoğaltıcı Wheatstone köprüsü adı verilen dörtlü bir direnç sistemine bağlı olarak çalışmaktadır. Dirençlerden biri sıcaklık sensörü olarak ayarlanmış ve diğer üç direnç referans alınarak aradaki fark cihaza beslenmektedir. Cihazın

kalibrasyonu için kullanılan direnç değerleri 2 kohm olarak ayarlanmıştır. Ayrıca cihazın çalışma şemasında cihaza bağlı başka bir direnç bulunmaktadır. Yapılan hesaplamalar sonucu direnç değeri 4.5 kohm olarak ayarlanmıştır. Sistem, kendi içinde sahip olduğu devre dizaynı sayesinde sözde bir toprak değeri ayarlayarak başlangıç çıkış voltajının 2.5 V olarak kalmasını sağlamaktadır. Yapılan denemelerde başlangıç çıkış voltajı 2.49-2,5 V değerleri arasında değişmektedir.

1. INTRODUCTION

The last century, microfluidic systems which are the combination of several methods become the most powerful tool in life-sciences for diagnosis of infectious diseases, genetic disorders and genetic traits [1]. There are several applications on microfluidic systems, but molecular cloning applications on molecular cloning promises to examine the reasons of several diseases at molecular level with using low volume samples [2]. Although the studies on molecular cloning have been accelerated after the invention of DNA polymerases1 and creation of recombinant DNA fragments, the quality and quantity of DNA which is obtained from the sample can still be a common problem. Nucleic acid amplification is a novel technique in life-sciences to gain enough amount of DNA to allow the experimental studies for molecular cloning [3].

First nucleic acid amplification is Polymer Chain Reaction (PCR) which is based on the replication of DNA by using Taq polymerase from E.Coli and six or more primers which are small RNA fragments to help replication with a thermal cycle which consists of heat denaturation and replication steps. Taq polymerases are thermostable polymerases, it means that they can protect their 3D structure at higher temperature. While the PCR process, DNA is denatured at 95°C to allow the access of DNA polymerase and replicated at 65°C, then the cycle is repeated [4].

After the success of the PCR, several amplification methods have been invented such as nucleic acid sequence-based amplification (NASBA), self-sustained sequence replication (3SR), strand displacement amplification (SDA). New techniques promise to eliminate the heat denaturation steps with innovation to re-initiate new rounds of DNA synthesis. For example, NASBA and 3SR use a set of transcription and reverse transcription reactions to amplify the target sequence and SDA employs a set of restriction enzyme digestions and strand displacement DNA synthesis with modified nucleotides as substrate. Although the efficiency of these methods are similar, still have shortcomings to overcome [5].

1.1 Purpose of Thesis

The main purpose of the thesis is creation of a new integrated mirofluidic chip that is able to amplify in very low amounts of DNA that is extracted from M. tuberculosis which has multidrug resistivity. Loop mediated isothermal amplification reaction is chosen for amplication of DNA and temperature stability is the key factor of the system. Because of that, the microfluidic chip is integrated with a microelectronic system to kept the temperature at 65°C within 1°C differences. Thesis focused on the fabrication and characterization steps of microfluidic chip and then integration with the prepared microcontrol system.

1.2 Literature Review

In this chapter, the working principle, key elements and literature overview of Loop Mediated Isothermal Amplification (LAMP) are introduced. Then, the applications of LAMP which is integrated to microfluidics chips in general are summarized, and then Tuberculosis which is the target microorganism of our experiment is presented. At last, the applications of LAMP on Tuberculosis in general and in the microfluidics chips are summarized.

1.2.1 Overview for microfluidic systems

Microfluidic is a new field to study fluidic behaviours with micron level technology as microchips which contain microchannels and miniaturized components such as tunnels, valves and chambers. Fluidic behaviour alters depends on volume of a liquid. In microfluidics, volume of a fluid is very small such as at femtoliter (fL) levels which is equivalent to quadrillionth of a liter. Fluids whose volume is so small behave quite different than macro scale. Therefore physics behind the fluid flow should be considered according to the volume of the fluid. This difference provides to open up a new horizon for new scientific studies [6].

Main idea behind the need of microfluidic study is to combine each equipment used in common laboratories in a one, simple integrated microsystem. Main component of a microchip is microchannels which can be produced by molding and engraving on surface of a microchip. Microchannels are networked structures onto microfluidic chips which is incorporated to environment by inlet and outlet holes with different dimensions. Fluids are injected through the inlet holes and evacuated from the outlet holes in microfluidic chip.

Fluid flows can be direct, mixture or manipulated to high-throughput systems. In this point, design of microchannel network is highly significant and shoul be considered precisely to achieve aim of study for instance lab on a chip systems, detection of microorganisms as pathogens, DNA applications as extraction, replication and many more [7].

Special systems are significantly needed to control fluid flows inside the microchannels. These systems can be embedded as quake valves or integrated by outside as pressure controllers [8].

Physical and chemical properties of fluids as gases and liquids are altered and developed by microfluidic systems in a manner of contrubition of technology. They have several benefits compared to conventional systems in macroscale. Microfluidic systems provide accurate analysis with less sample volume therefore required materials as chemical, reagents are reduced globally. They provide shorten time as fast measurement and lots of operation can be conducted on a single chip due to compact size [9].

Microfluidic system also offers devastating parameter control and high quality data obtaining without performance decreasing. Since sample volume is too low, handling of analysis and processing are much more easier than conventional macroscale systems. In microfluidic systems, each microchip can be used easily without expertise need also complex reactions with multiple processes can be performed with high level of functionality. The microfluidic systems are utilized for different applications such as analysis of DNA sequences to detect toxins or printing by inkjet method and many more [10]. Microfluidics systems have broad range of advantages to conventional systems: preventing time consuming due to low reaction time, high sensitivity, portability, precise temperature control, easy automation, integration of equipments in common labs in to one simple lab on a chip device.

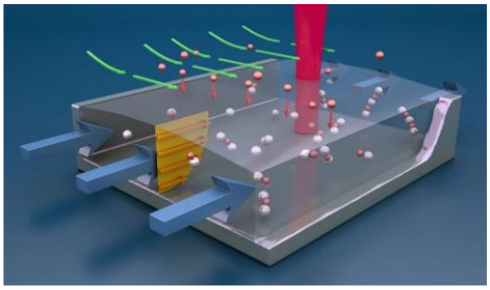


Figure 1.1. An example for microfluidic systems [11]

Although it contains lots of microsclae components as integrated whole system, it is cost effective. In recent studies, microfluidic systems are used as developed tools in diverse research fields and especifically in biological sciences such as [12]:

- Easy and fast analysis due to less reaction time.
- Sample consumption is decreased due to small sample volume.
- End user analyses all processes integrated and simplified.
- Cost effective
- High and multiple throughput
- Portable system for point of care devices
- High accuracy and resolution for measurement

1.2.2 Loop mediated isothermal amplification (LAMP)

Loop mediated isothermal amplification is a novel, high-sensitive and cost-effective method to amplify nucleic acid under isothermal conditions [13]. It is invented by a group of scientists from Eiken Company and published at 'Nucleic Acids Research', in 2000 Jun 15. Two inner and two outer primer, a target DNA which contains six different regions from 5' end and Bst DNA Polymerase are required for the system[14]. Bst polymerase is another prokaryotic thermostable 5′→3′ exonuclease isolated from the thermophilic bacterium Bacillus stearothermophilus (Bst). The optimum temperature of Bst Polymerase is defined as 65°C, and high Mg2+ concentrationis essential for maximum activity. Also it can be inactivated after 15 min incubation at 75°C Protease digestion of Bst DNA Pol I generates two protein fragments. The large protein fragment of Bst DNA Pol I is thermostable, and thus very useful for sequencing reactions performed at 65°C to avoid problems due to hairpin formation. Like Klenow, the Large Fragment of Bst DNA Pol I shows a faster strand displacement than its full length counterpart. Recombinant Bst DNA Pol I is presently available from Epicentre Biotechnologies [15,16]. The advantages of the LAMP reaction can be ordered as [17]:

i. DNA is amplified via LAMP method with high efficiency under isothermal conditions without a significant influence of the co-presence of non-target DNA.

The detection limit of LAMP is a few copies and its can be accepted as effective as PCR.

ii. LAMP is only requires a DNA polymerase, fewer primers compared with PCR, and a regular laboratory water bath or heat block for reaction, so it can be defined as easy, simple and cheap.

iii. The specificity of LAMP is provided by six independent sequences in the initial stage and by four independent sequences during the later stages of the LAMP reaction for the target sequence.

iv. LAMP can also amplify RNA sequences with high efficiency by combination with reverse transcription method2.

v. Pyrophosphate ions are produced as a by-product from the LAMP reaction substrate deoxyribonucleotide triphosphates (dNTPs) while the DNA amplification process by DNA polymerase, The calcein is combined with manganous ion (Mn2+) to be stable at initiation part and then manganous ion is deprived of calcein by the generated pyrophosphate ion (P2O7 4_) when LAMP reaction occurs and it results in the emission of fluorescence. Then, the free calcein combined with magnesium ion (Mg2+) in the reaction mixture, so that it strengthens the fluorescence emission.

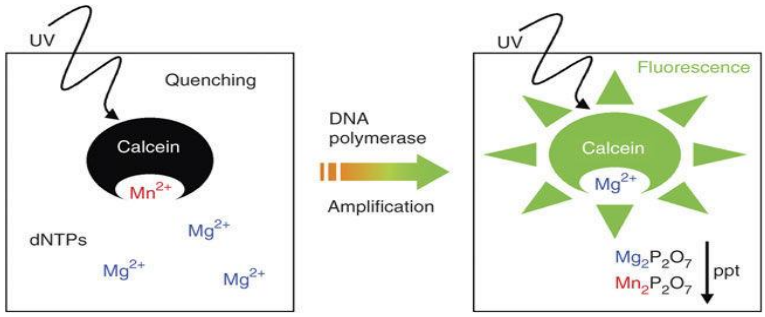


Figure 1.2 Principle of detection using a fluorescent metal indicator (calcein) [18]

The reaction mechanism can be seen at Figure-1.2[18].The process of LAMP consists of three common steps: Sample preparation which consists of the template of DNA, Reaction and detection of amplification. An example for the process can be seen at Figure-1.3 [19].

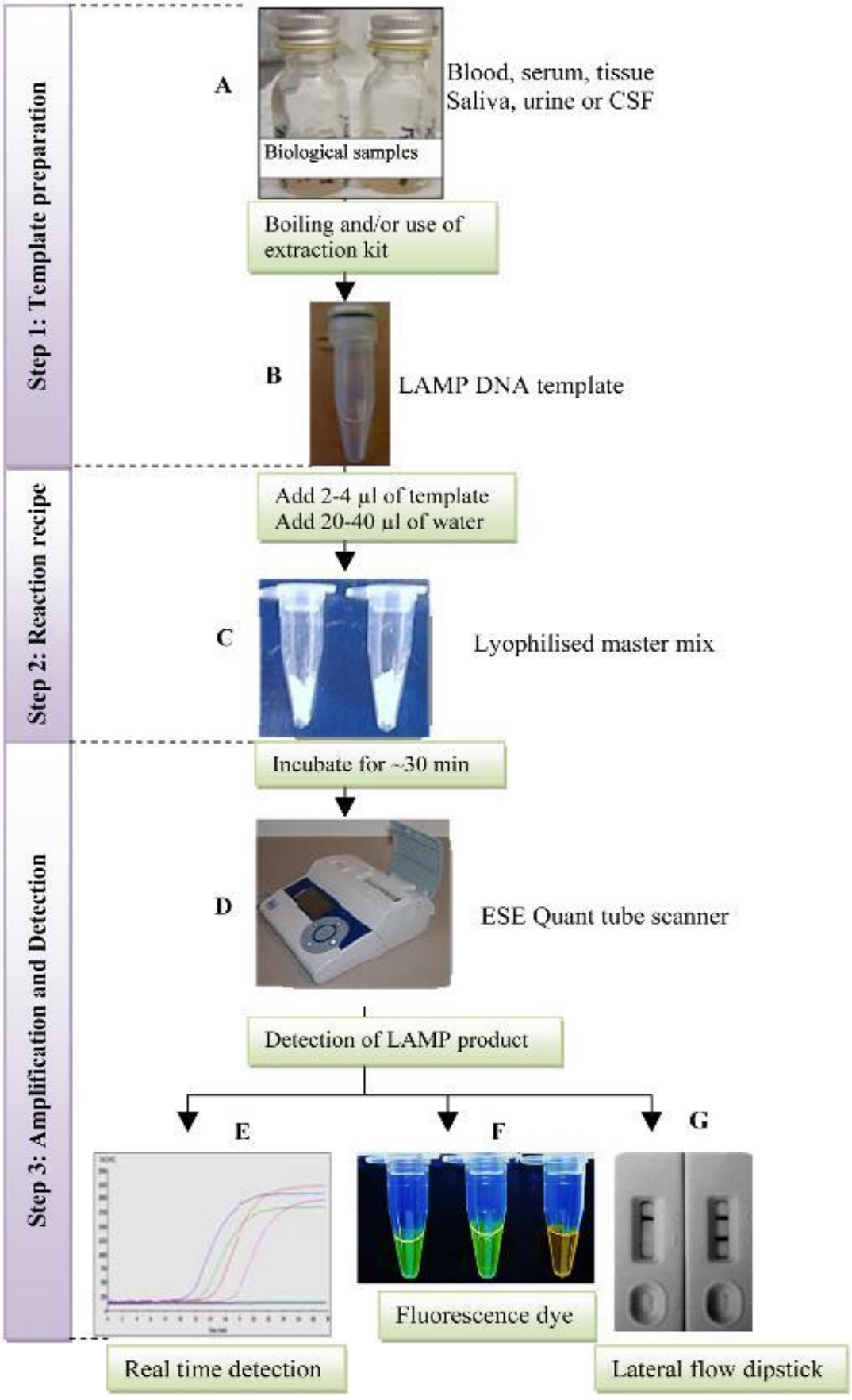


Figure 1.3 A proposed three-step LAMP method for diagnosis of neglected tropical diseases.

1.2.3 Loop-mediated isothermal amplification integrated on microfluidic chips

The application of DNA-type analyses in specific microfluidic systems become common to minimise sample processing and handling. For example, whole blood samples can be analyzed for direct PCR analysis within microfluidic channels.

Microfluidics technology has also illustrated a potential to be allied with the detection of very low numbers of DNA molecules, i.e. potentially individual molecules. Foquet et al. [20] have shown that the construction of fluidic channels of <1 μm enables the detection and relative proportions of mixtures of DNA molecules to be measured. In addition, using an electrical field to control the flow rates analysis times of only several milliseconds per DNA molecule become achievable.

Electrophoretic mobility shift assays for the detection of DNA-protein interactions have also been carried out in a microfluidic chip environment [21]. Some of the benefits achieved are reduced sample volumes, an avoidance of labelling procedures and decreased analysis times.

1.2.3.1 Literature review

In the literature, various experiments are performed on different pathogens which are summarized at Table-1 and it can be seen that the reaction can be successful even the volume of DNA is a few nanoliters .

Table 1.1. The summary of different experiment which are performed LAMP reaction on different pathogens [22-29]

Method	Material	Amplicon	Volume	Detection
LAMP	Silicon	Virulence genes (various, 20 min)	50 µL (10 chambers)	Turbidity and SYBR Green
	PDMS	λDNA (48,502 bp input, 70 min)	2 µL (for nL droplets)	Calcein
	PDMS/Glass	Virulence genes (various, 60 min)	30 nL droplets	EvaGreen
	PMMA	Pathogenic bacteria (various, 30 min)	48 µL (1.414 µL/reaction well)	Gel + SYBR Green (off-chip)
	PMMA	*Salmonella* (-, 70 min)	25 µL	SYBR Green
MDA	PDMS	*E. coli* (whole genome, 10–16 h)	60 nL	SYBR Green (off-chip)
	PDMS	MCF-7 cells (whole genome, 16 h)	1.4 nL	qPCR (off-chip)
HDA	Polymeric	BRCA1 gene (113 and 157 bp, 15 min)	35 µL	Gel
RCA	Glass	16 S rDNA (various, 110 min) off-chip amplification	30 µL	SPR-Biosensor
	Glass	OLR1 gene (-, 30 min)	500 nL	EvaGreen
RPA	Various	*E. coli* ($bla_{CTX-M-15}$ gene, 15 min)	270 nL	Cy5 labeled probes
	DVD-R discs	Various (-, 2 h)	3 µL (sample volume)	Optical density
	PMMA/Glass	2 viruses and 1 bacterium (142, 144 and 181 bp, 48 min)	54 µL	Luminol

An example of microfluidic chip which consists of a PDMS reaction chamber which is integrated with a pneumatic pump and gold microheater can be seen at Figure-1.4.

The system is designed for detection of The nervous necrosis virus via RT-LAMP reaction to produce complementary DNA from the virutic DNA [30].

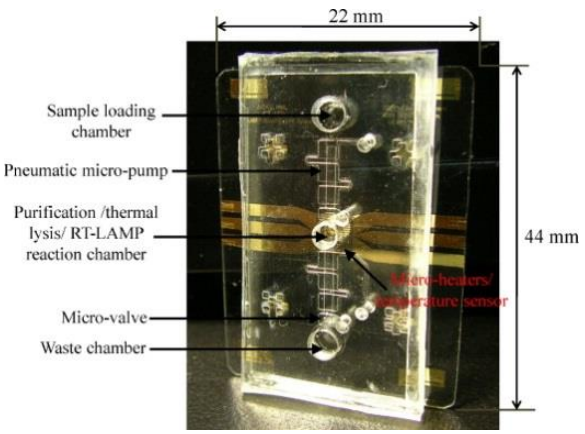


Figure 1.4 An example of microfluidic chip which consists of microheater and reaction chamber parts [30]

The LAMP systems are digitalized to detection of the absorbance value of fluorescence which is released during the LAMP reaction occurs. Rean et all developed a novel technique to detect several pathogen by adopted LAMP reaction inside the microfluidic chip[30]. The illustration of the microfluidic channel can be seen at Figure-1.5.

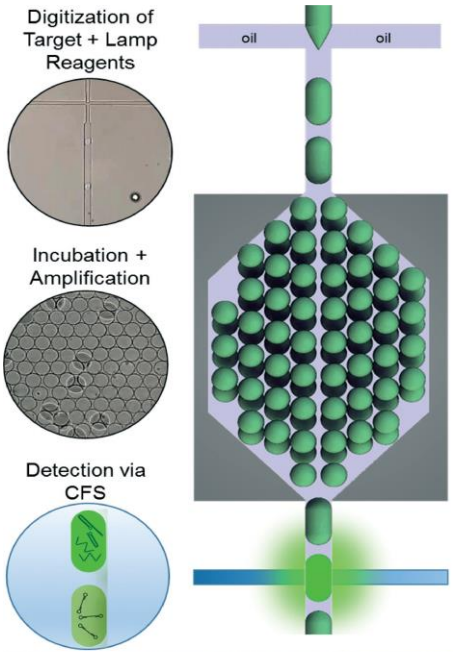


Figure 1.5 Detection of LAMP reaction via fluoresence spectrum [31]

1.2.3.2 Commercial LAMP systems

Microfluidic systems have been utilized for simplification of high-throughput biological assays At China's National Center for Nanoscience and Technology (NCNT). An eight-channel microfluidic LAMP test has developed that the naked eye or an optical sensor can detect the result. Not only the chip promises to provide simplicity, but also it solves other problems of users. For instance, lab technicians can be sick due to the released aerosol from the LAMP reaction, the LAMP reaction completely occurs in microfluidic channels, and it eliminates exposure to this hazardous byproduct [32].

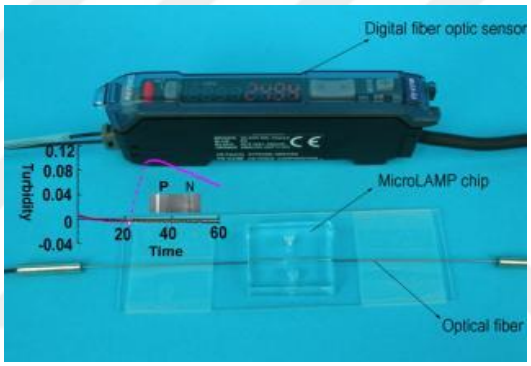


Figure 1.6. A commercial LAMP system with an optical sensor for detection of the turbidity or fluorense material [32]

1.2.4 Tuberculosis

Tuberculosis is an airborne infectious disease which can affect bone, the central nervous system and many other organs but especially attacks to the lung alveolar surfaces[36]. Tuberculosis is the oldest and most fatal disease which can be spread from person to person into the aerosol droplets through the air[33]. It is reported that 10.4 million people was infected and 2 million people died around the world in 2015, though the widespread use of an attenuated live vaccine and several antibiotics [34]. Tuberculosis is caused by an acid-fast bacteria called *Mycobacterium tuberculosis* that is the member of the *Mycobacterium* family whose peptidoglycan structure is ropelike. Although other species of *Mycobacterium* can live in soil and water, *Mycobacterium tuberculosis* is mostly defined as pathogen that can choose the human lung cells as host cells [35-37].

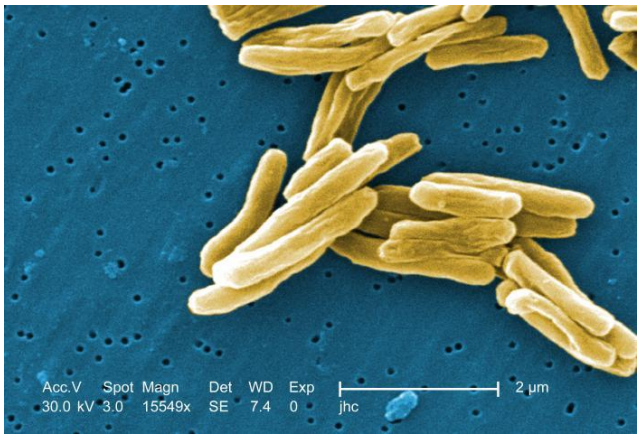


Figure 1.7. Under a high magnification of 15549x, this digitally-colorized, scanning electron microscopic (SEM) image depicted a number of Gram-positive Mycobacterium tuberculosis bacteria. As an obligate aerobic organism M. tuberculosis can only survive in an environment containing oxygen. This bacterium ranges in length between 2 - 4 microns, and a width between 0.2 - 0.5 microns [38].

The Mantoux tuberculin skin test and abnormal chest x-ray are the common diagnosis techniques which are used to indicate the TB infection.Although the morbidity rate of TB has decreased in many developed countries, has been rising in developing countries. One of the reasons for failure to control TB in developing countries is the lack of affordable simple diagnostic methods that have better sensitivity than the sputum smear test commonly used in the resource-limited rural laboratories [39,40].

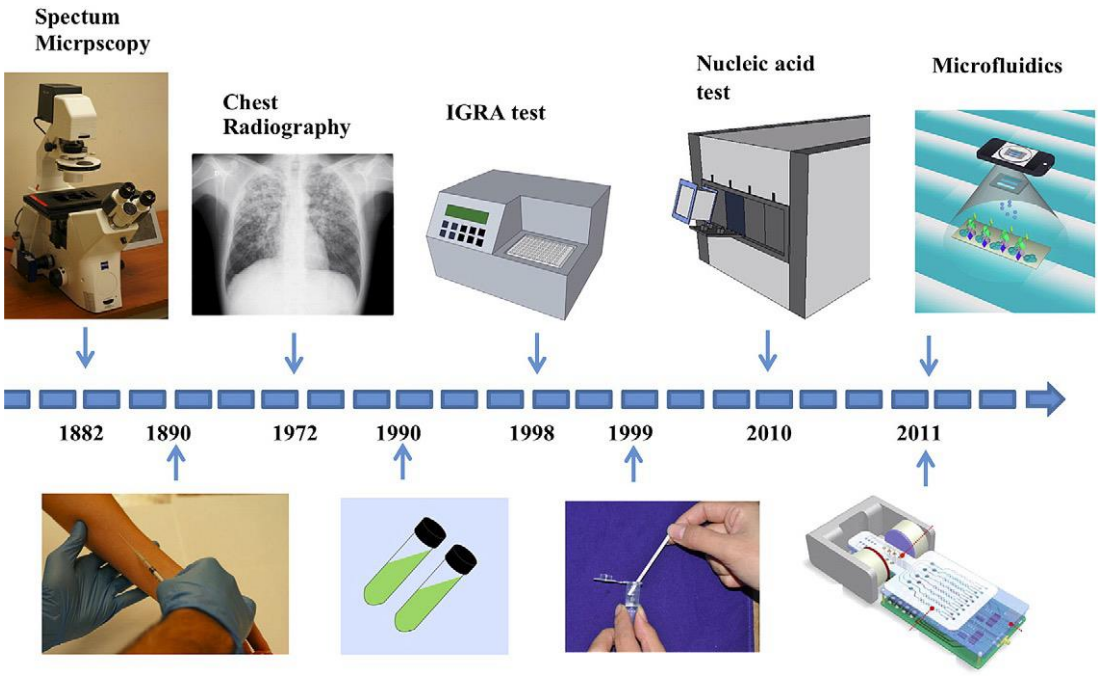


Figure 1.8. The history and evolution of the diagnosis techniques for Tuberculosis

1.2.5 The applications of LAMP reaction on *Mycobacterium tuberculosis*

Although NAT systems for TB have become common in developed countries, there is no NAT system that is simple enough and affordable enough to be acceptable in the rural laboratories of developing countries. Iwamoto et al. have reported a LAMP assay that can detect *Mycobacterium tuberculosis* as well as *M. avium* and *M. intracellulare*. This assay has almost the same sensitivity as that of the PCR based Amplicor system (Roche Diagnostics GmbH: Basel, Switzerland) which tests sputum samples decontaminated by N-acetylcycteine-NaOH treatment. Boehme et al. have reported the evaluation of a LAMP assay designed for use in peripheral microscopic laboratories in developing countries. In their study, the LAMP method with a filterbased sample pretreatment method for raw sputum was demonstrated to have enough simplicity for use in resourcelimited settings and to have almost the same clinical performance as that of commercialized NAT for TB.

Eiken Chemical which is founded at 1939 in Japan is the biggest player for the investigation, production and commercialize the enzymes and reagent kits for immunochemical, clinical chemistry, molecular genetics, medical devices and industrial products. They represented a novel LAMP reagent kit for detection of the M. Tuberculosis complex (Loopamp MTBC detection kit, TB-LAMP; Eiken Chemical, Tokyo, Japan) in April 2011. Two common problems for TB-LAMP systems are sputum processing and shell life of the reagents which are essential for LAMP reaction. The new reagent kit promises to resolve them and make the process easier and faster. First improvement is Loopamp PURE DNA extraction kit (Eiken Chemical) which consists of the NALC (N-acetyl-L-cysteine)-NaOH decontamination step for sputum processing. Second improvement is the usage of dry reagents for TB-LAMP to allow easier storage, even stored at room temperature with satisfactory shelf life. The steps of operation process of PURE and TB-LAMP is demonstrated at Figure – 1.9. Both systems were examined to investigate their efficiency and sensivity from the researchers. According to Mitarai et al., PURE-TB-LAMP is a simple, effective, and rapid test for TB test and the sensitivity of TB-LAMP for smear negative and culture-positive samples is about 55 % [41,42]. Bost systems can promise a rapid and effective detection method for Tuberculosis where the expensive equipments and chemicals are not available.

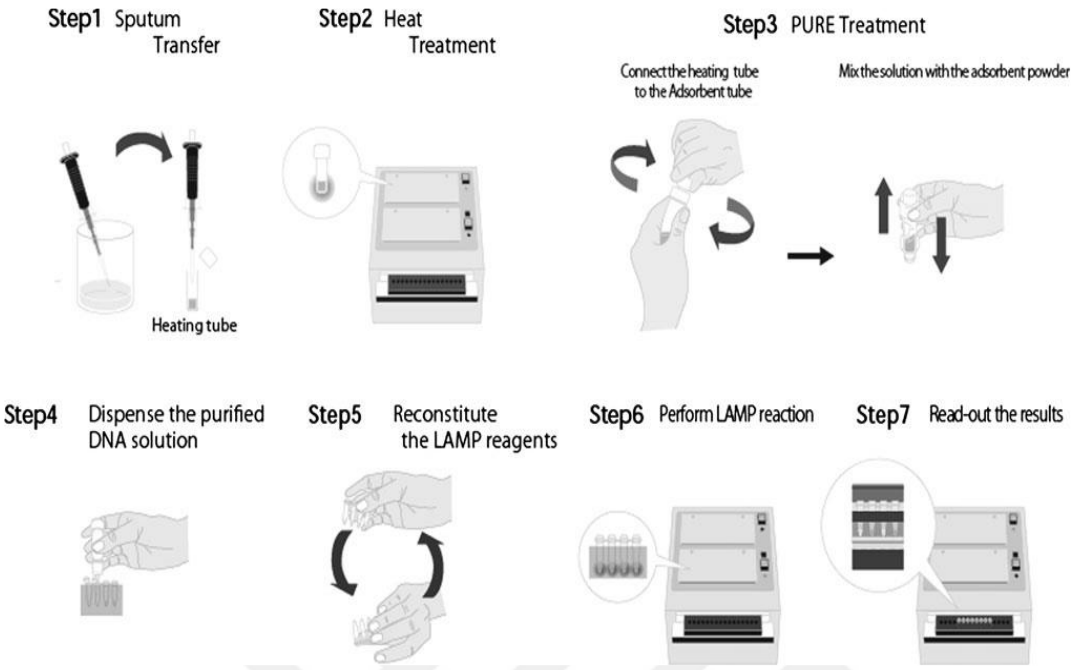


Figure 1.9Steps of TB-LAMP extraction Kit

2. DESIGN & MODELLING OF MICROFLUIDIC CHIP

In this chapter, the design of heating element and reaction chamber as the parts of microfluidic chip are described in general terms and the results of different versions of the design which were modelled by a Finite-Element Method based simulation program are demonstrated.

2.1 Materials of the Experiment

2.1.1 Heating element

The material of heating element was chosen as Alumininum thanks to its features such as high electrical and thermal conductivity, rapidly heating behaviour and being biocompatible. The substrate was chosen as glass due to its low electrical and thermal conductivty, biocompatibility and being available for Al coating. The physical properties are available at Appendix-A

2.1.2 Reaction chamber

The reaction chamber was made by PDMS which is silicone elastomer stable thermally, has high permeablity to gases, manufactured and manipulated easily, exhibits isotropic and homogeneous properties as well as lower cost than silicon, and can conform to submicron features to develop microstructures. In addition, PDMS is opaque, biocompatible, non-fluorescent and nontoxic. All these features make PDMS attractive for the development of MEMS and microfluidics components for biomedical applications has been traditionally used as a biomaterial in catheters, drainage tubing, insulation for pacemakers, membrane oxygenators, and ear and nose implants with desirable properties that. PDMS can be shaped via using Soft Lithography techniques such as micro-contact printing, replica molding, micro-transfer molding, micro-molding in capillaries, and solvent-assisted micro-molding.

2.2 Design of Microfluidic Chip

Microfluidic chip consists of two main parts: a heating element and a reaction chamber. The heating element is designed to heat the reaction chamber rapidly and keep the temperature constant throughout the reaction. The heating element which can be named as microheater, is surrounded by a temperature sensor for measurement of the temperature of microheater. Design of microheater and temperature sensor are taken from an article whose title is "Design and fabrication of a quasi-ordered nanoporous silicon membrane suitable for thermally induced drug release"[43]. At the article, three different structures which are meander, spiral and coil are illustrated and their illustrations are demonstrated at Figure- 2.1.

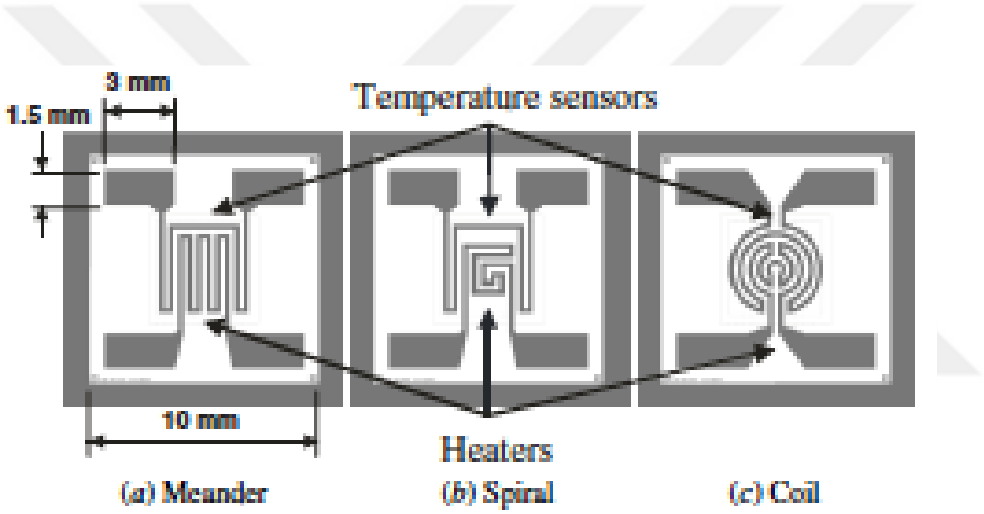


Figure 2.1 Three different structure which are designed as heating element which consists of microheater and temperature sensor

The reaction chamber is designed to transport all heat which is produced from microheater uniformly and designed to obtain the suitable area where the reaction can take place. For PDMS based platforms, the main problem is evaporation of water and diffusion through its porous structure. The structure consists of three stages which are designed to reduce the evaporation of water by creating a heating block at the centre of the chamber. . The structruce contains one input to fulfill the chamber with the mixture solution and one output to transfer the reaction solution after reaction finishes. The alternative designs whose length is varied between 3 mm to 5 mm is demonstrated at Figure-2.2

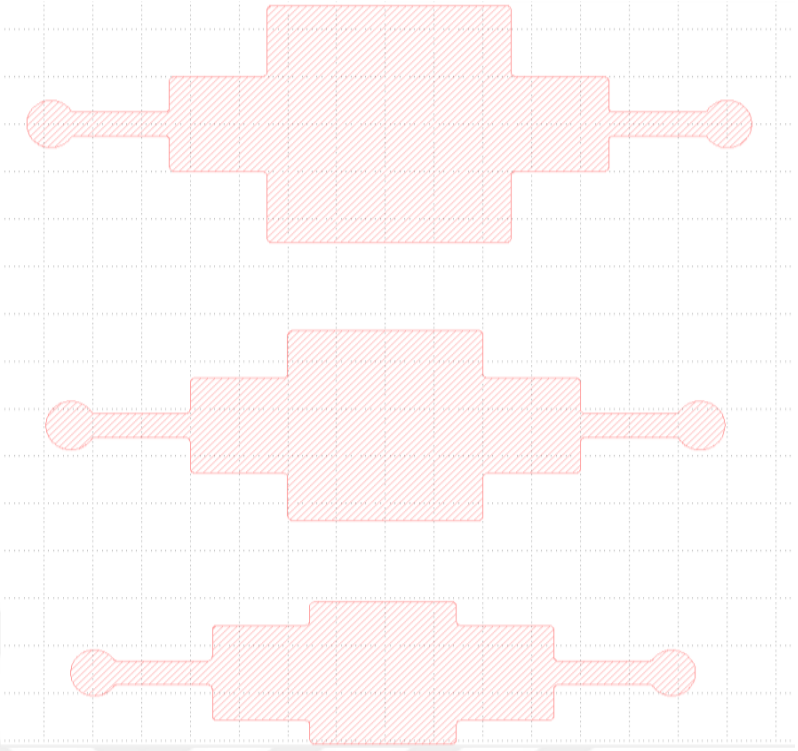


Figure 2.2Three different design of PDMS based reaction chamber. The length of the chamber is adjusted 3,4,5 mm.

2.3 Modelling of the Microheater on COMSOL

All of the structures were analyzed for investigation of temperature distribution by using a finite-element analysis (FEA) simulation tool, COMSOL. The 3D simulation was performed by combination of Joule heating and shell, conductive media, DC modules to study on the transfer of electrical heat generation by getting rid of geometrical constraint. The properties of the materials, the additional information about the structure and the physics can be provided from Appendix-A.

At first, the effect of the geometry on temperature distribution was examined for all structures and the results are demonstrated at Figure-2.3 .

According to the results, the heat generation is focused at the centre of the structure and some energy losses occur between the layers. Because of that, the distance between the lines are decreased from 100 µm to 70 and 20 µm The FEM results to examine the effect of decreasing the distances on uniformity of temperatureis demonstrated at Figure-2.4.

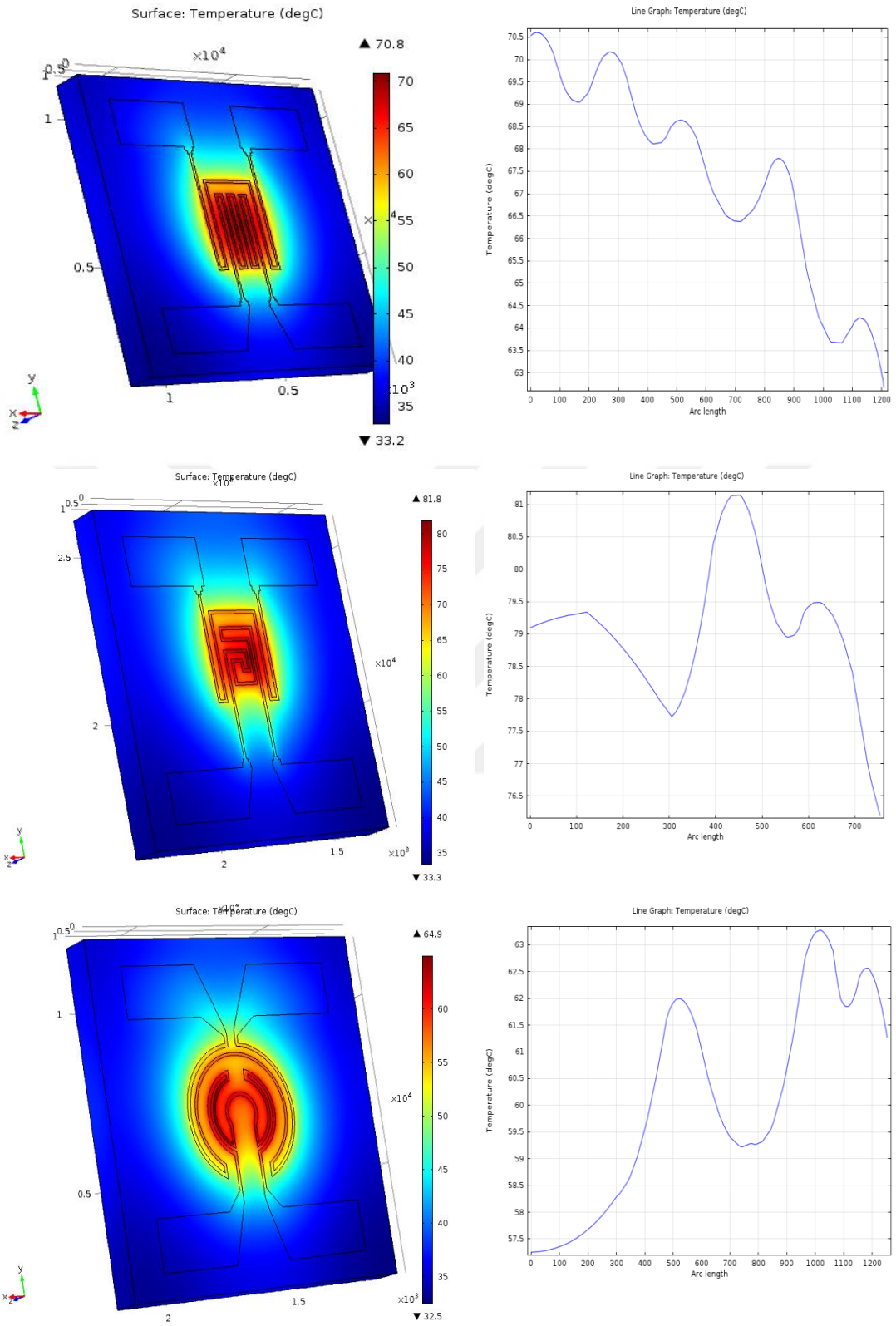


Figure2.3. The FEM results and the graphs of temperature distribution on the microheater

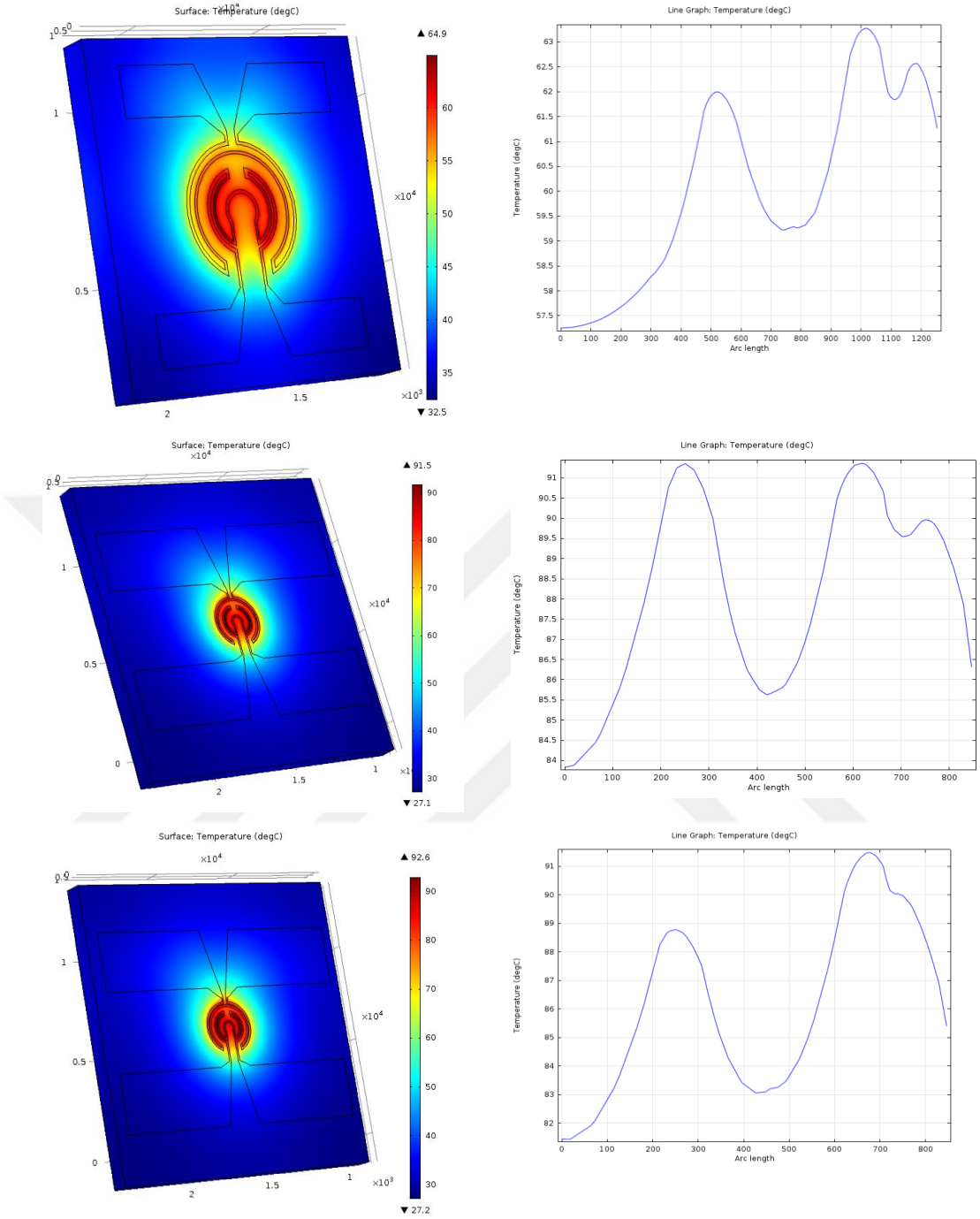


Figure 2.4 The FEM results which demonstrated the effect of the distance between the Al lines

Although the energy loss can be minimized by decreasing the distance between the Al lines, the heated area is getting smaller. Also, the maximum temperature for the system become higher than our target temperature even studying at low voltages and it may cause to control of temperature via microcontrol system. Because of that, the distances between Al lines are adjusted as 100 μm.

For protection of heating element and avoid the interaction of biomolecules and coating, the heating element was planned to coat with SU-8 which is a special polymer. Also, The positively effect of SU-8 coating to increase the uniformity of heating distribution was prescribed and the heat distribution and energy loss were examined according to the thickness of coating.

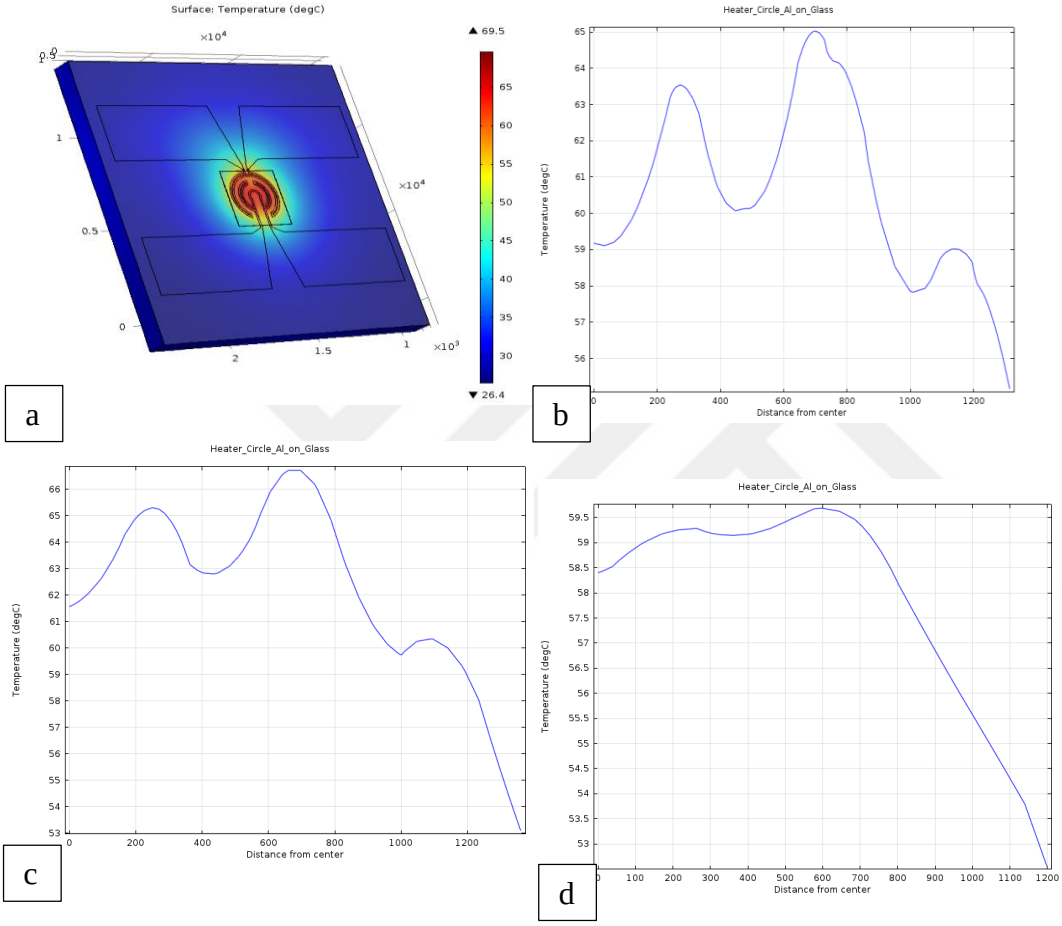


Figure 2.5 a. The FEM result of SU8 coated Al microheater b. The result of 15 um SU8 coated Al microheater c. The result of 30 um SU8 coated Al microheater d. 50 um SU8 coated Al microheater

The uniformity of heat on the heater increased with increasing the thickness of SU8, but energy loss increases, too. For a significant effect on heat uniformity, the thickness of SU8 should be higher than 50 um. Because of that, the thickness of SU8 was adjusted as 15 um.

3. FABRICATION OF MICROFLUIDIC CHIP

The microfluidics chip whose design is presented at previous part was fabricated at Nanoresearch Center and MEMS laboratories. Microfluidic chip consists of two main part: a heating element and a reaction chamber.

3.1 Equipments

3.1.1 Photolithography

Photolithography which is demonstrated at Figure- 3.1.a is the main equipment for fabrication of both structures. The first step of lift-off process which is available at Appendix-B and the preparation of PDMS mold were performed on photolithography and the masks which were used on the photolitography were prepared with The Heidelberg DWL 66fs mask writer which is demonstrated at Figure- 3.1.b.

Figure 3.1. a. SUSS MA10 Mask Aligner b The Heidelberg DWL 66fs mask writer.

The masks were prepared with the semi-glass 5 inch photomasks which are coated with chrome and the mold of PDMS were fabricated on 4 inch silicon wafers. Both substrates are demonstrated at Figure -3.2 .

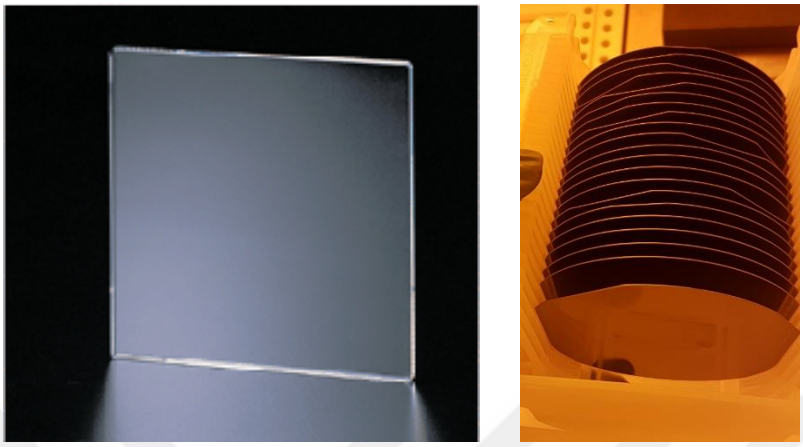


Figure 3.2. a. 5 inch blank chrome photomask b. 4 inch blank silicone wafer

3.1.2 Spin coater

Spin coater which is demonstrated that Figure-3.3.a was used to coat different photoresists to prepare the molds for fabrication of the structures. Developing and curing of the photoresists steps were performed at wet bench which is demonstrated at Figure-3.3.b. The wet bench consists of a spin coater, a baker, an ultrasonic equipment and bench is connected with a nitrogen tank with a gun to dry the samples and a reservoir for collection of residues.

Figure 3.3. a.Spin coater b.Wet Bench

The steps for cleaing the substrate and coating of photoresists on glass substrate was applied on wet bench. Acetone, alcohol and distilated water are the common chemicals for cleaning steps.

3.1.3 Thermal evaporation coating system

The aluminium coating to prepare the heating element on the glass substrate was performed on Vaksis thermal evaporation coating system which is a type of physical vapor deposition technique. The material is placed into a boat and the boat is heated by Joule heating phenomena. The material is evaporated due to the heating and coated onto the substrates which are located on the top of the chamber. The thermal evaporation coating system is demonstrated at Figure – 3.4 .

Figure 3.4. Vaksis Multicoating System

3.1.4 Circuit board plotter

The Printed Circuit Board (PCB) which the microfluidic chip is placed on and the electrical connections are made is designed via CircuitCAM and fabricated via Protomat S100 Circuit Plotter. The plotter consists the drilling and milling functions to shape the copper coated FR-4 plates.

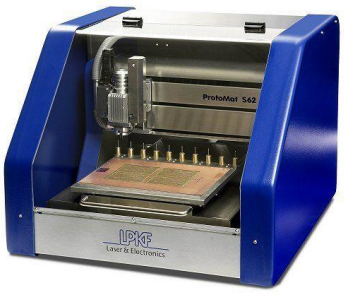


Figure 3.5. Protomat Circuit Plotter

3.1.5 Wire bonder

The thickness of the microheater and temperature sensor was not available to apply solder to make electrical connections, because the operation temperature of solder caused to damage the coating. Therefore, Wire Bonder which is a common technique in microchip industry is used to make electrical connections between the pads of heating element and printed circuit board. The working principle of wire bonder is based on disruption of the metal wire by ultrasonic force, diffusion of the metal atoms through the atoms of the PCB and evolution of an intermetallic surface[44].

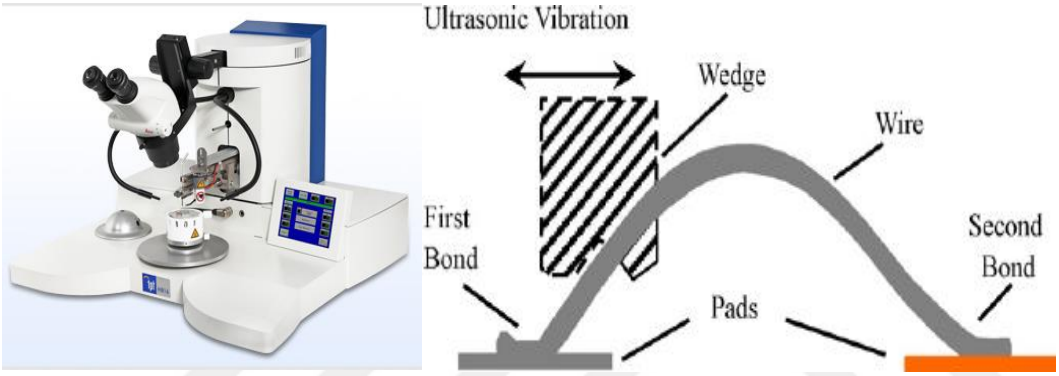


Figure 3.6. a. TPT50 Wire Bonder b. The illustration of wire bonding process

For wire bonding process, two main application types can be identified: ball bonding and wedge bonding. The bonding types are named due to the shape of the ends of the bonds on the pads. For ball bonding, the material is degraded by applying high voltage and prepared a ball, then the ball is mounted on the PCB by applying force to allow the diffusion of the atoms of wire metal through the atoms of PCB for the determined time on the first pad, and then the degraded metal is pasted on the second pad and table teared. Generally, gold and aluminium are chosen as wire metals; copper becomes new player nowadays. The the most important parameter for chosing the right material is the thickness of the pads. Despite the high resistance to oxidation and high electrical conductivity of gold, the thickness of aluminium must be higher than 1 µm to form an intermetallic surface[44]. Because of that reason, the wire metal whose thickness is 33 µm has chosen as aluminium. For aluminium wire, wedge bonding is available which is demonstrated at Figure -3.7.

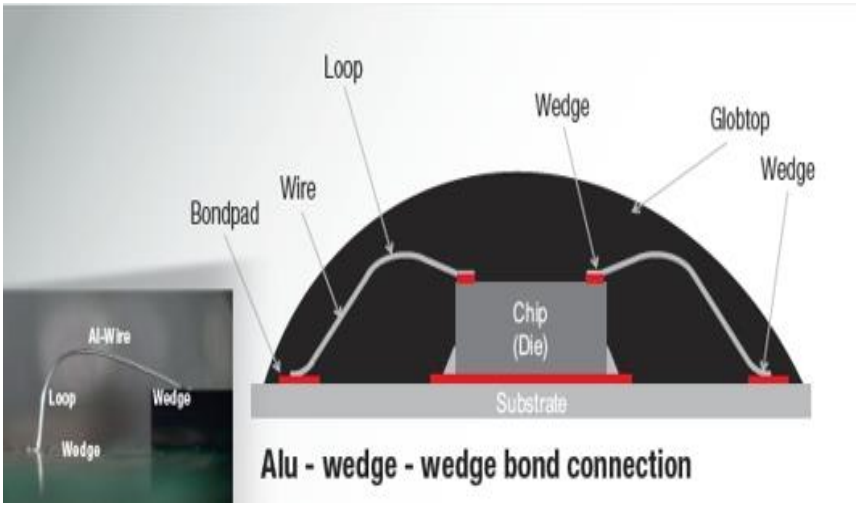


Figure 3.7. The illustration of Al wedge bonding [45]

3.2 Fabrication of the Microfluidic Chip

3.2.1 Heating element

The heating element which consists of microheater and integrated temperature sensor was fabricated on glass substrate whose dimensions are 15 mm x 15 mm x 1 mm via lift-off technique. The procedure of lift-off technique and the dimensions of the heating element are available at Appendix B-1. The structures were incorporated at the center of the mask; a rectangle and a circle for fabrication of the SU-8 layer on the structure are incorporated on both sides of the structures. The sketch of three different design of the heating element and the image of the prepared chrome photomask are demonstrated at Figure -3.8 .

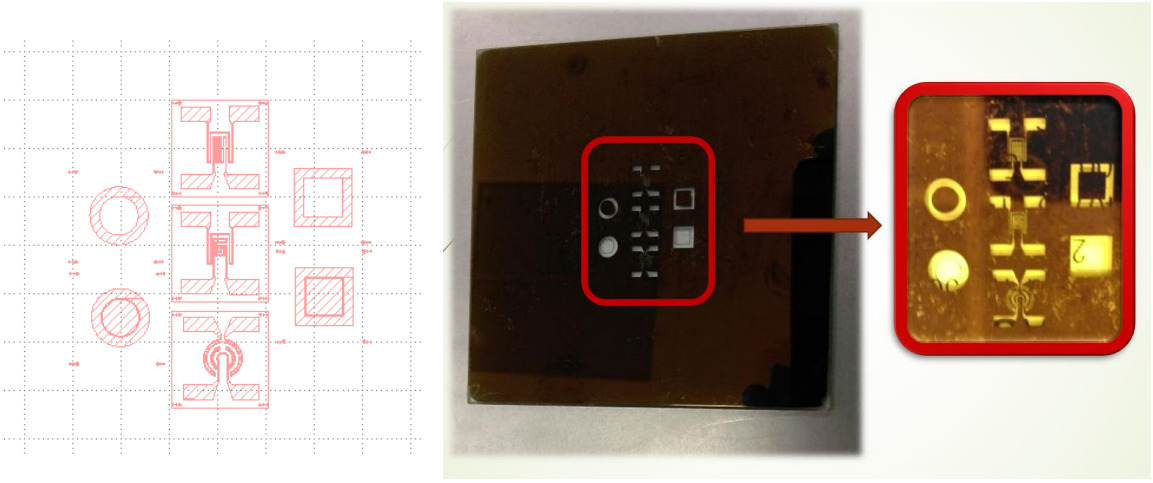


Figure 3.8. Three different design of the structure of integrated microheater and temperature sensor are shown.

200 nm and 300 nm Al coated microheaters are demonstrated at Figure-3.9 . They will be packaged and integrated with the microcontrol system after fabrication part.

Figure 3.9. The fabricated heating elements

3.2.2 Reaction chamber

Reaction chamber was prepared from PDMS and the procedure of PDMS mold preparation and PDMS pouring is available at Appendix B-2. Three different structures were prepared to reduce the evaporation of the liquid inside of the chamber. The mold for PDMS and its image under the microscope are demonstrated at Figure-3.10 .

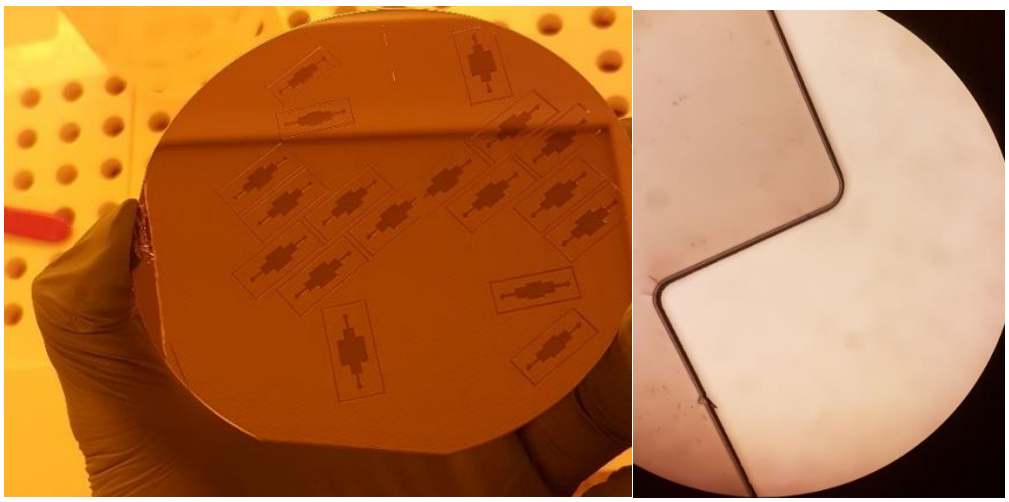


Figure 3.10. The mold of reaction chamber and its image under the microscope

The molds were prepared by photolithography and the height of the chamber was adjeusted as 100 μm.

3.2.3 Electrical connections of microfluidic chip

The microheater of the system can be accepted a long tiny resistance and is designed to be heated by applying enough current. Also, the resistivity of temperature sensor changes due to the amount of heat which is produced by microheater and the resistivity change will be the basic of the microcontrol system. Not only heating the system but also integration of heating element and microcontrol system, the electrical connections of both structures were prepared via wire bonding technique. The design and product of temporary PCB for measurements are demostrated at Figure-3.11 . The temporary design consists of four pads which will be connected with the pads of the heating elements.

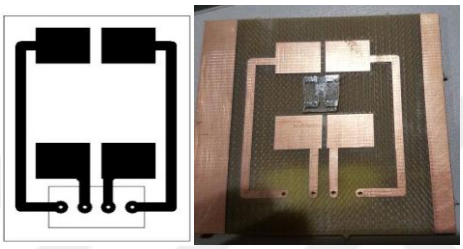


Figure 3.11. The design of temporary printed circuit board and prepared system

The parameters which are required for Al wedge bonding are available at Appendix-C. The material of the first pad is 300 nm Al coating and the material of second pad is copper coating from PCB. Different examples of wedge bonded samples and the final product is demonstrated at Figure-3.12 .

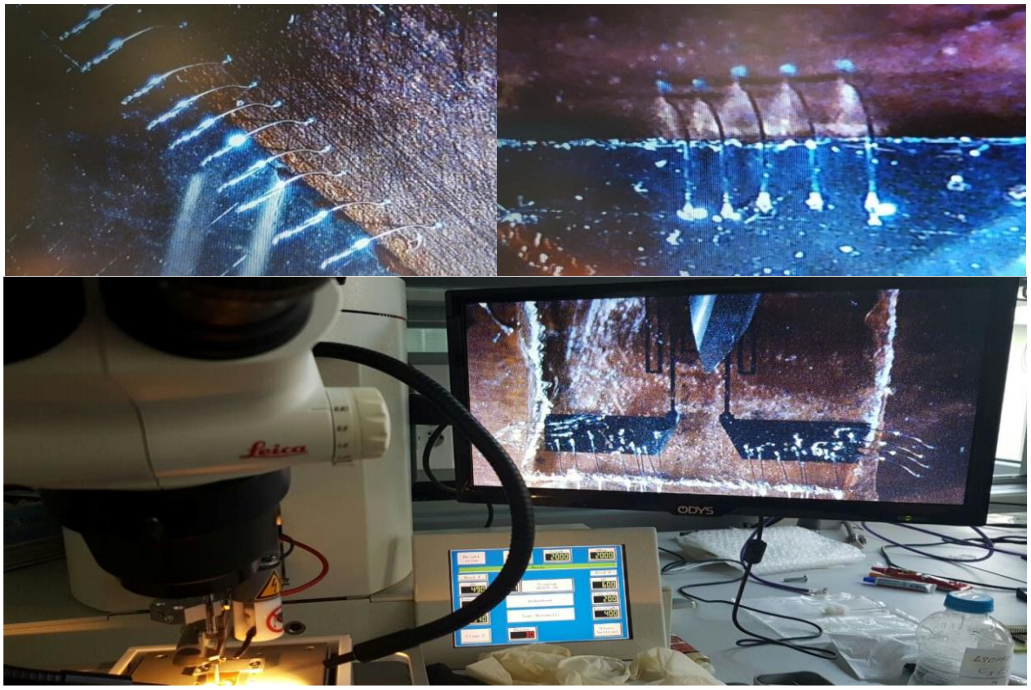


Figure 3.12. Wedge Bonding on Al coating samples

4. CHARACTERIZATION OF MICROFLUIDIC CHIP

In this chapter, the resistiviy and temperature measurements of microfluidic chips are demonstrated. The microelectronic system will be designed based on sensing the resistance difference of Al lines as temperature sensor due to heating by microheater.

4.1 Equipments

4.1.1 Probe station

After fabrication of the heating element, the resistivity of both structures was measured by four-point probe which is demonstrated at Figure -4.1 . The probes are connected to Karl Suss PA300 model probe station and the chuck, the area which the samples are placed, can be worked between -60°C and 200 °C. The probe statin was purchased from Class I Equipments and it is placed at VLSI laboratory.

Figure 4.1. The four point probe station

4.1.2 Power supply

The microheater and microelectronic system has been worked by an adjustable power DC Power Supply which is purchased from Agilent. The power supply consists of two modes which indicates two different ranges of voltage values. Low mode indicates the voltage values between 0-8 V with 3 A as maximum current value and high mode indicates 0-20 V with 1.5 A as maximum current value. The power supply consists of two outputs and both of them can work together.

Figure 4.2 DC Power Supply

4.1.3 Semiconductor parameter analyzer

The resistivity of the temperature sensor was measured via Hewlett Packard 4155A model Semiconductor Parameter Analyzer. Two probes were connected to the device and one probe worked as positive side and other probe worked as ground. The value of electrical potential was adjusted between 0 to 0.5 V with 0.05 steps and the current values of the temperature sensor were measured.

Figure 4.3 Semiconductor Parameter Analyzer

4.1.4 Thermocouple

Temperature measurements were taken by using a temperature controller which is connected with coupled conductive wires who has their different thermal expansion rates [45].

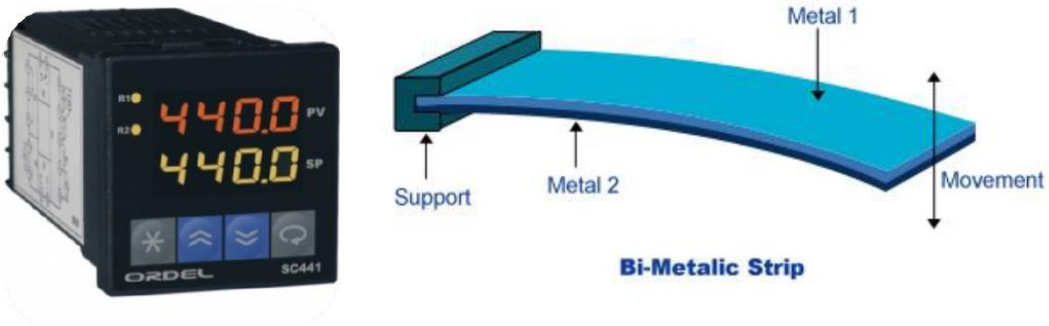


Figure 4.4 The temperature controller and the illustration of a bimetallic thermocouple [45]

4.2 Measurement of the Resistivity of Microheater and Temperature Sensor

Two probes were connected with DC power supply to apply the voltage to the microheater. Other two probes were connected with analyzer and measured the current values to be taken from the temperature sensor when voltage is applied to the coating in steps of 0.05 volts between 0-10 volts at different temperatures which is required by microheater.

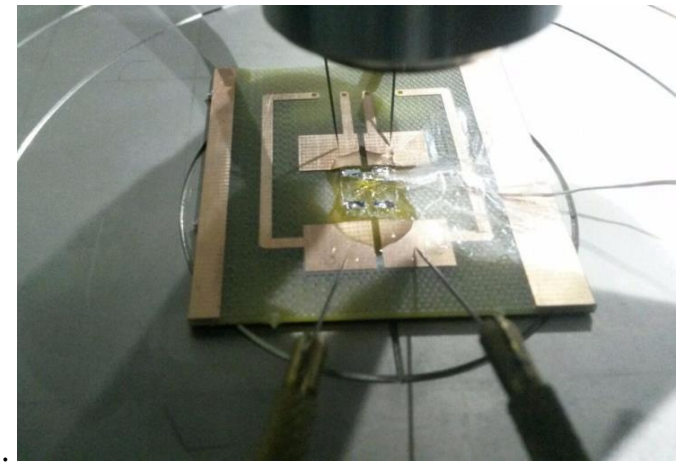


Figure 4.5 Test setup on probe station for measurement of the resistivity of microheater and temperature sensor

The resistivity measurement was performed for every steps of fabrication part and the results are demonstrated as graphs. Because of some problems during the fabrication

part, the spiral microheater design was cancelled and other designes were characterized.

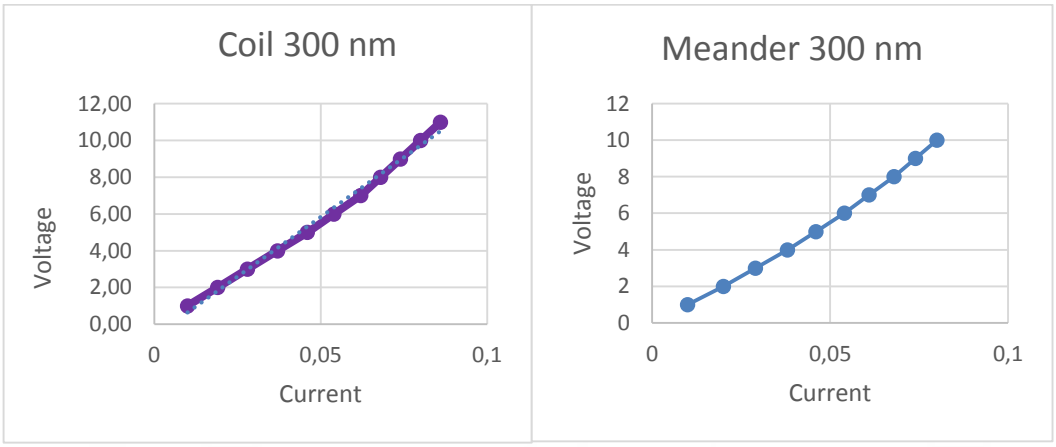


Figure 4.6. The graphs which demonstrates the increase of temperature due to the increase of the voltage for both structure

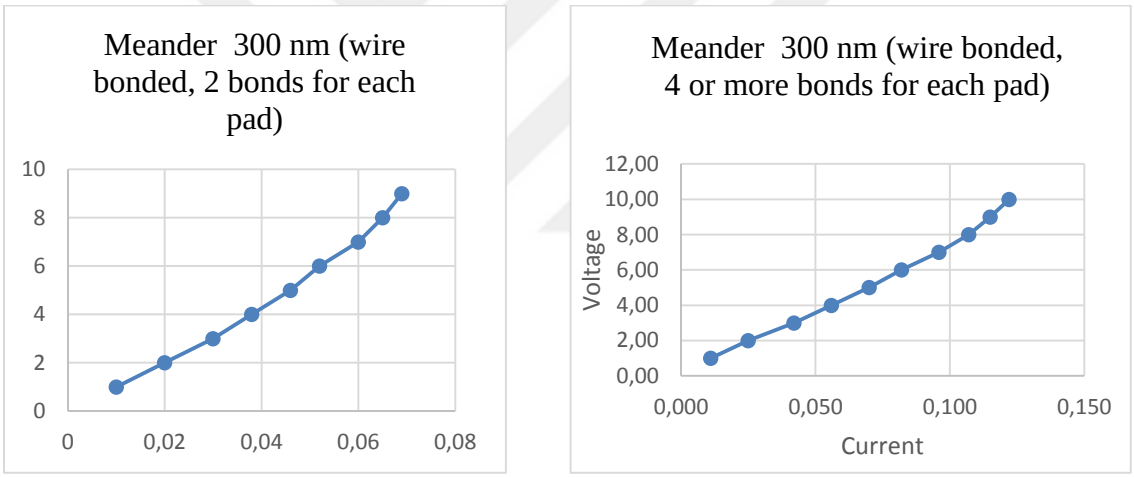


Figure 4.7. After electrical connections, the resistivty values were measured to characterize how the number of the wire affects the resistivity

Limitations of the system:

- Maximum voltage value which can be applied to the microheater is 12 V for 300 nm Al coating.

- The Al wire cannot carry higher values than 120 mA.

- The Al coating cannot carry higher values than 80 mA.

4.3 Temperature measurement of microheater

The same test set-up was used for temperature measurement and the results are given as graphs at Figure-4.8 . The temperature of heaters were measured between 1-10 V by increasing 1 V.

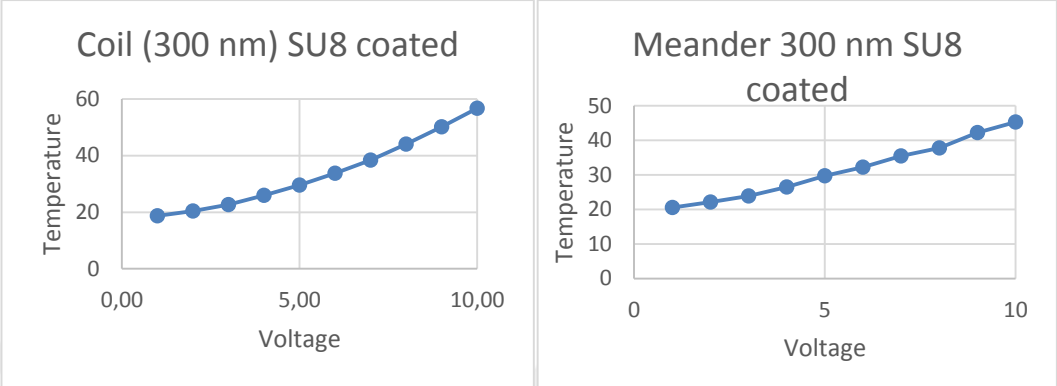


Figure 4.8 The temperature measurement results of both structure which are coated with 15 um SU8

According to the measurements, the behaviour of coil shaped microheater is consistent with the literature, but the meander shape must be optimized. The temperature sensor is made of aluminium and its resistivity increases due to heating which is released from microheater. During temperature-resistivity measurements of microheaters, the resistivity values of temperature sensor was measured at several temperatures. While the heater was set on a temperature, the temperature sensor was connected to semiconductor analyzer and a voltage whose values are between 0-0.5 V by increasing 0.05 V was applied via semiconductor analyzer. The measurement results for meander shaped were given at Figure-4.9.

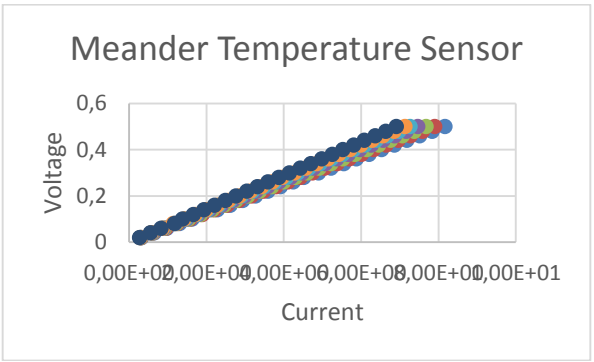


Figure 4.9. Resitivity measurements of temperature sensor at several temperatures.

The resistivity values for each temperature was calculated from the slopes of the lines which indicate the voltage-current relationship. The temperatures and resistivity values of temperature sensors are demonstrated at Table-4.1.

Table 4.1. The resistivity values of temperature sensor for several temperature values

Temperature	Resistivity
29	61,25
36,1	63,21
43,7	65,08
54,4	67
64,5	68,59
75,8	70,1
86,7	72,45

At last, the power consumption of the microheater was calculated according to Equation (1). The power consumption due to heat generation can be seen at Figure-4.10.

$$\text{Power} = I^2 * R = V^2/R \qquad (1)$$

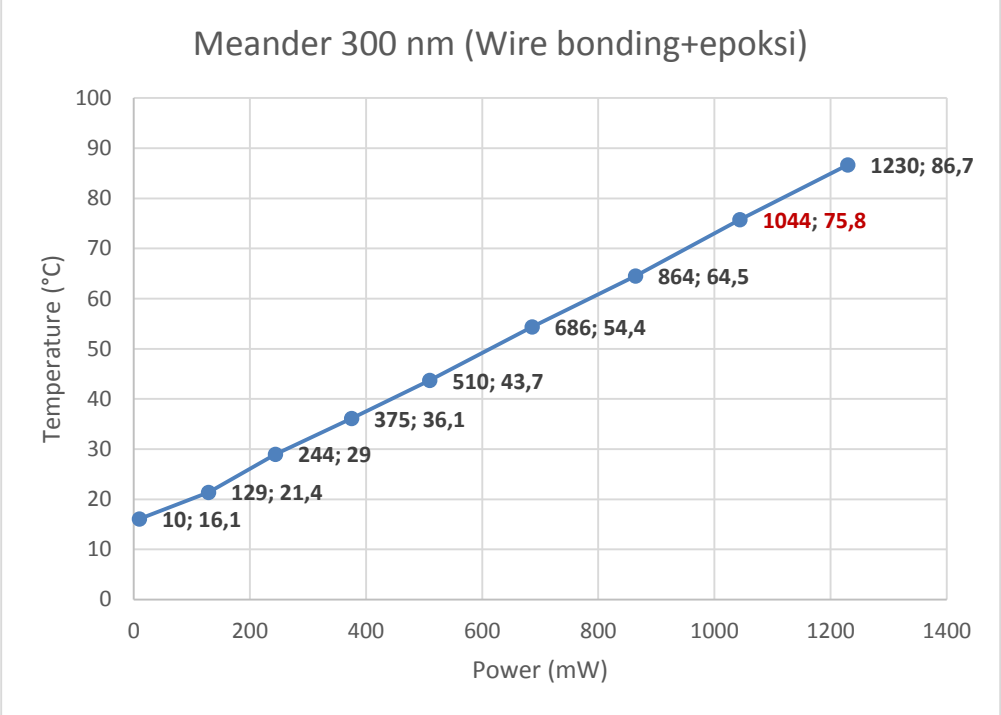


Figure 4.10 Power consumption values of microheater at several temperatures. The values are calculated from Equation (1)

After electrical connections were done, temperature measurement was applied independently. The new test set-up is demonstrated at Figure-4.11 .

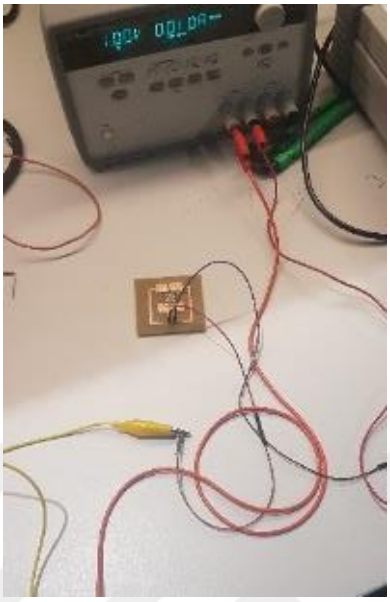


Figure 4.11. The prototype of microheater which is connected with DC power supply

The results of independent prototype can be seen at Figure -4.12 . It can be observed that the microheater can catch the target temperature around 7 V.

Voltage	Current	T (ºC)
1	0,01	27,7
2	0,02	31
3	0,03	34,2
4	0,038	38
5	0,046	45,7
6	0,052	56,8
7	0,06	69,7
8	0,065	78,1
9	0,069	84,2

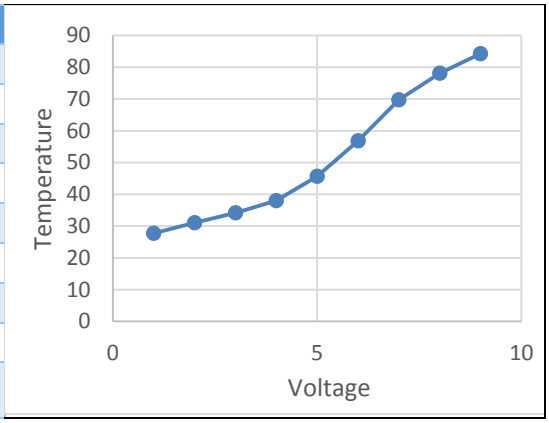


Figure 4.12. The temperature measurement result of the independent prototype

5. DESIGN OF MICROELECTRONIC CIRCUIT

5.2 Key Elements of Microelectronic Circuit

5.2.1 Arduino

Arduino is an open-source electronic platform which contains Atmega328 microcontroller to be ready for programming by a user-friendly software. Figure-5.1 demonstrates Arduino Uno and the schematic of electronic platform [46].

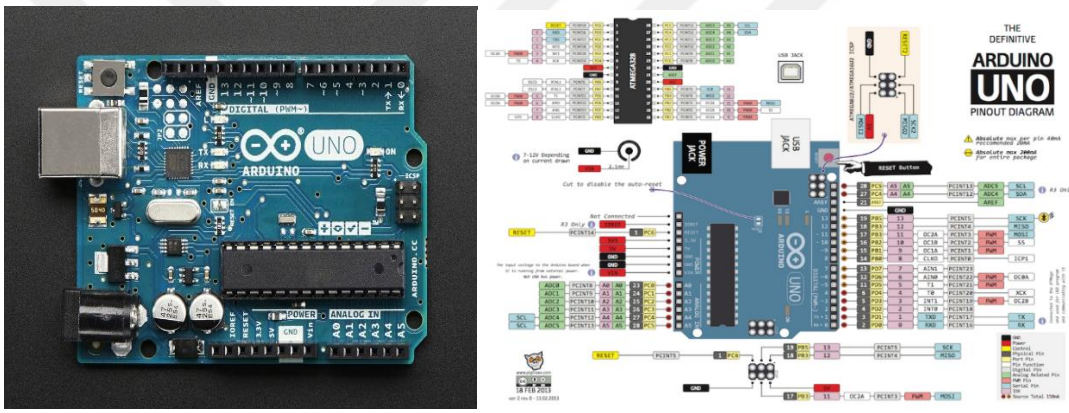


Figure 5.1. Arduino is an open source microcontroller package

5.2.2 INA125 amplifier

The INA125 is a low power commercial instrumentation amplifier which promises high accuracy with a precision voltage reference by complete bridge excitation and precision differential-input amplification on a single integrated circuit. A Wheatstone bridge is integrated to the amplifier to gain from 4 to 10,000. The INA125 can be operated on single (+2.7V to +36V) or dual (±1.35V to ±18V) supplies with low offset voltage (250mV), low offset drift (2mV/°C), and high common-mode rejection (100dB at G = 100). The voltage reference is externally adjustable with pin selectable voltages of 2.5V, 5V, or 10V, allowing use with a variety of transducers. The INA125 can be protected with 16-pin plastic DIP and SO-16 surface-mount packages and is available to operate from –40°C to +85°C industrial temperature range [47].

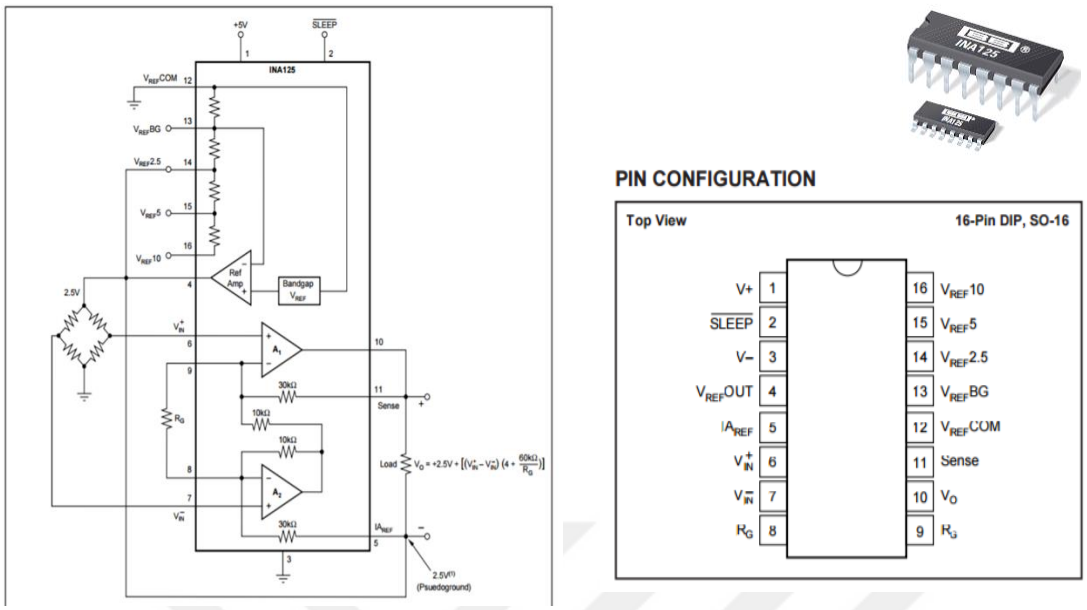
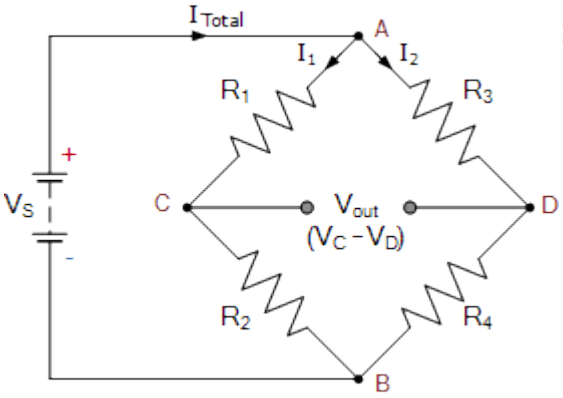


Figure5.2. The schematic, pin figuration and real image of INA125

5.2.2.1 Wheatstone bridge

Wheatstone bridge is a combination of resistances to detect the value of the resistance due to the voltage differences between the two points of the system. The system and equations of the Wheatstone bridge are demonstrated at Figure-5.3 [48].



$$V_{OUT} = (V_C - V_D) = (V_{R2} - V_{R4}) = 0$$

$$R_C = \frac{R_2}{R_1+R_2} \quad \text{and} \quad R_D = \frac{R_4}{R_3+R_4}$$

$$\text{At Balance: } R_C = R_D \quad \text{So, } \frac{R_2}{R_1+R_2} = \frac{R_4}{R_3+R_4}$$

$$\therefore R_2(R_3+R_4) = R_4(R_1+R_2)$$

$$R_2R_3 + \cancel{R_2R_4} = R_1R_4 + \cancel{R_2R_4}$$

$$\therefore R_4 = \frac{R_2R_3}{R_1} = R_X$$

Figure 5.3. Wheatstone Bridge

5.2.3 Transistor

The NPN transistor is an electronic system piece that is designed to pass electrons from the emitter to the collector. The emitter “emits” electrons into the base, which controls the number of electrons the emitter emits. Most of the emitted electrons are “collected” by the collector, which sends them along to the next part of the circuit[49].

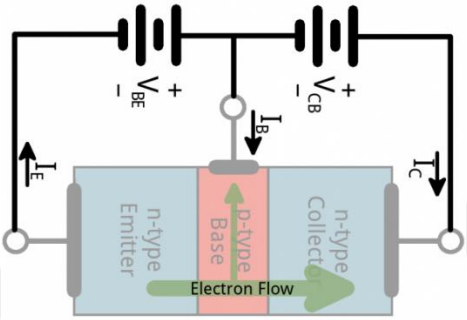


Figure 5.4. The working principle of the NPN type transistor

5.2.4 Breadboard

Electronics breadboards are one of the most fundamental pieces which allows to study on temporary circuits, integrated circuits or prototypes without solder process. **Prototyping** is the process of creation of a preliminary model and examination of its behaviour under the given set of parameters Breadboards are suitable platforms to build a prototype and test it out solderless[51]. The structure of breadboards and an example are demonstrated at Figure -5.5 .

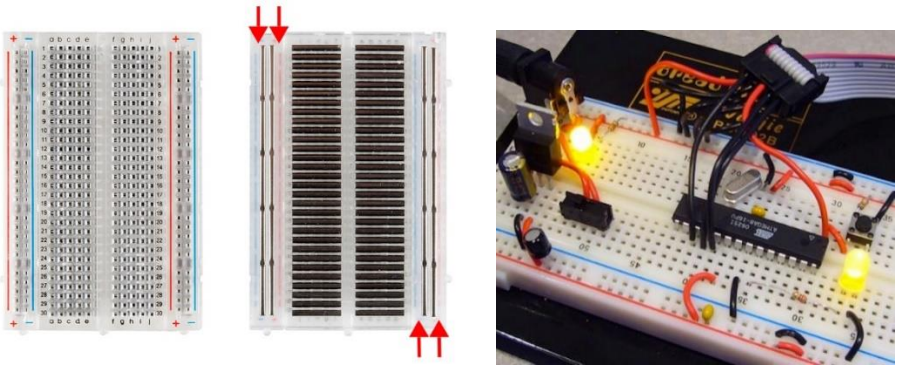


Figure5.5. a. The inside of the breadboard. The vertical and long metals are used to connect the power supply and ground parts of the prototypes; the horizontal and short metals are connect the leg of the circuits. b. An example of a circuit on breadboard[50]

5.2.5 Printed circuit board

A printed circuit board (PCB) is the common tool which is produced from copper sheets laminated onto a non-conductive substrate for connection of electronic components mechanically and electrically via conductive tracks, pads and other features. Components of the circuits (e.g. capacitors, resistors or active devices) are generally mounted on the PCB by soldering process or embedded for advanced PCBs. FR-4 glass epoxy is the insulation layer of PCB and conductive layers can be added on one or both sides. Also, the PCB can be coated multiple times with conductive materials especially copper and conductors on different layers are connected with vias. Multi-layer PCBs allow for much higher component density[51]. The structure of a double layer PCB can be seen at Figure -5.6 .

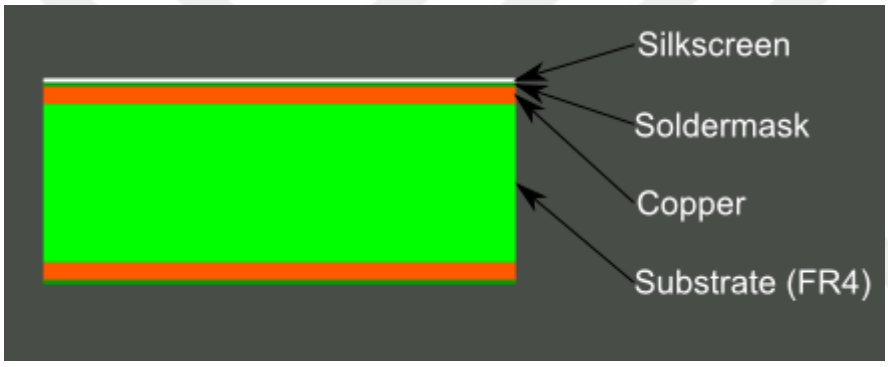


Figure 5.6. Schematic of Double layer Printed Circuit Board[52]

5.2. Microelectronic Circuit

5.2.6 Design of the microelectronic circuit

The microelectronic system is designed to control the temperature of microheater and to set it at predescribed temperature within 1oC difference which is required for the LAMP reaction. The microelectronic system consists of a microcontroller, an operational amplifier, a transistor, a regulator, lcd and keypad and the schematic of the circuit can be seen at Figure-5.7 .

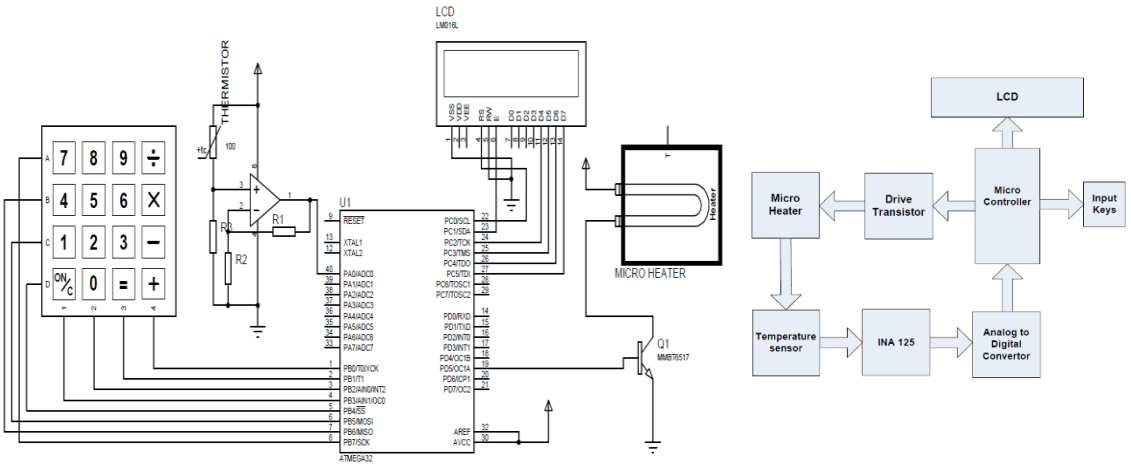


Figure 5.7. The schematic of the microelectronic system that will be integrated with microfluidic chip

The essential part of the microcontrol system is detection of the resistivity difference and giving an answer in seconds. The temperature sensor is connected with the Wheatstone Bridge that is a part of the amplifier. The resistivity of the temperature sensor increases due to the generated heat by microheater and that difference causes to a voltage response. The voltage value is fed to the microcontroller and compared with the reference value. If it is out of the range the current which passes from a leg of the transistor is cut. Other leg is connected with the microheater and it works as switch. The system has been studied on breadboard and its image is demonstrated at Figure-45 . Also, the programme is available on Appendix- D.

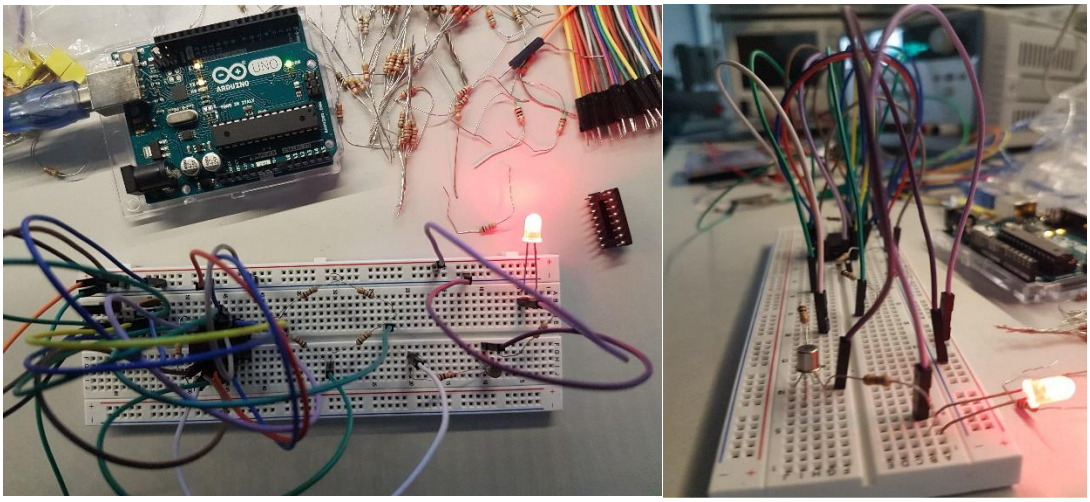


Figure 5.8. The microelectronic circuit on the breadboard

5.2.7 Design of printed circuit board

Printed Circuit Board was designed on Altium Designer 14.3 and the Gerber files which were taken from the program were used for fabrication. The program consists of two main steps: Preparation of the schematic for identification of the pieces of the circuit and demonstration of the connections between them, and then preparation of layout to replace every pieces of the circuit on the right positions. The schematic of our microelectronic system is demonstrated at Figure-5.9 .

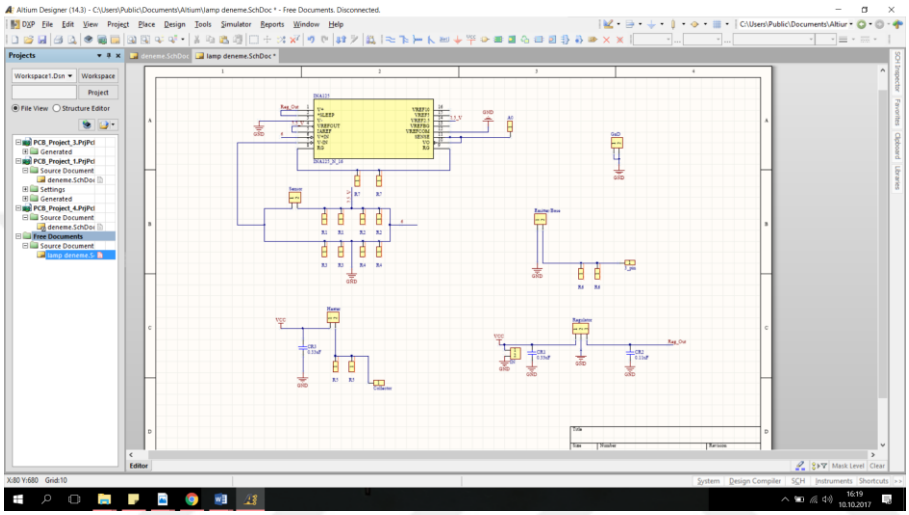


Figure 5.9. The schematic design of the electric circuit

The layout was prepared by combination of the footprints of the pieces which are defined at schematics part. The footprint of INA125 was added into the library of the program and other components were taken from the library of the programme. “C” indicates capacitors and “R” indicates to resistors. The layout of our system is demonstrated at Figure-5.10 .

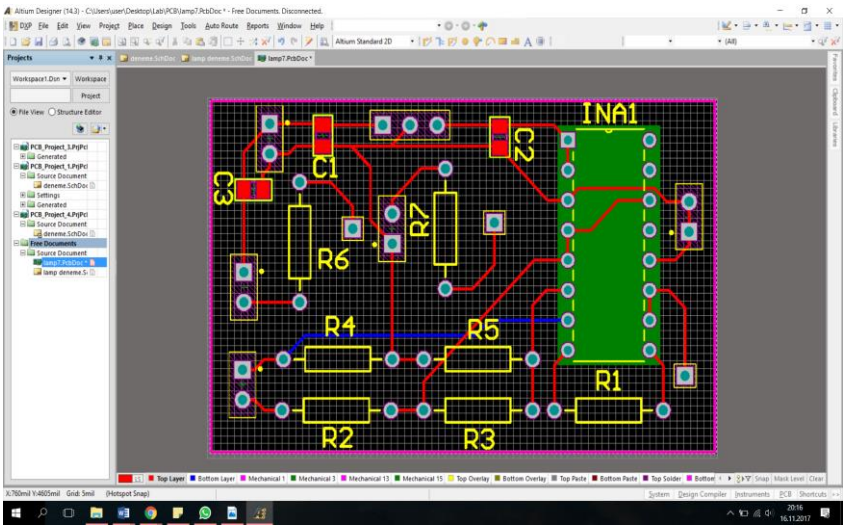


Figure 5.10. Design of the printed circuit board

6. INTEGRATION OF MİCROFLUIDIC CHIP AND MICROELECTRONIC SYSTEM

The integration of both systems part, two pathways were followed:

- The system was tested on breadboard to adjust the value of the resistances on the Wheatstone Bridge and other resistances connected with the transistor and microheater. The test set-up is demonstrated at Figure -6.1 .
- After adjusting the value of the resistances, the printed circuit board was prepared and fabricated. While test step of the system, the heating element and the the PCB were connected via headers and cables.

The program which is uploaded onto the microcontroller which is a part of the Arduino is available at Appendix-D. After programming step, the microcontroller can be stacked out from the platform and integrated into the designed microelectronic circuit. For optimization part, the dimensions of the PCB part of microfluidic chip will be getting smaller and integrated onto the microcontrol system. The new design of printed circuit board which consists of both structure and microcontroller is being studied.

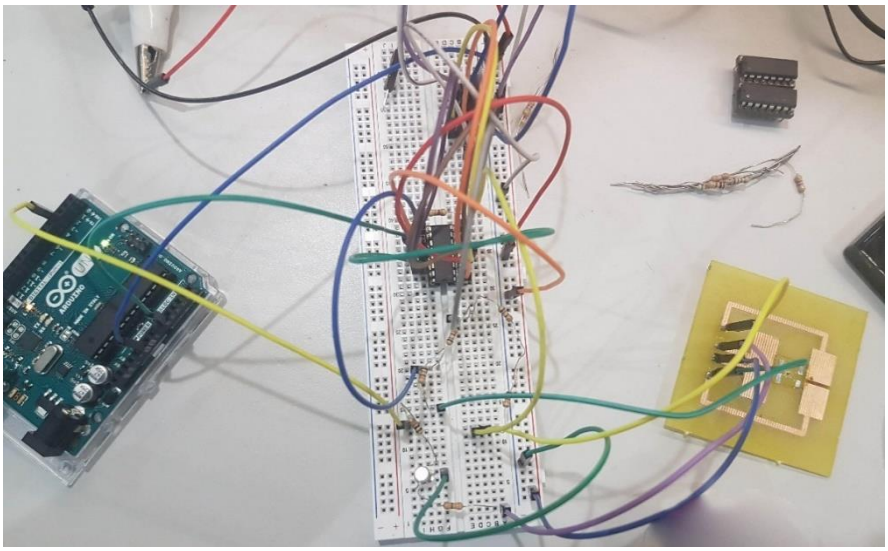


Figure 6.1. Integrated microcontroller system and microfluidic system

7. CONCLUSIONS AND RECOMMENDATIONS

A novel microfluidic system is designed for detection of M. tuberculosis and amplification of bacterial DNA via Loop Mediated Isothermal Amplication reaction. The reaction requires stable temperature while the reaction occurs and no heat denaturation step is included.

- Aluminium is a good alternative for fabrication of microheater, but another material with higher resistivity values can be chosen for temperature sensor. Because, detection of resistivity differences due to the heat generation from the microheater can be hard for low temperatures. This limitation may cause to decrease the sensitivity of the system.

- Soldering is main problem for microfluidic systems, because the thickness of coating is not resistant to high temperatures. Wire Bonder can be a good alternative to make the electrical connections.

- 300 nm is the suitable thickness value for our system due to some errors about the fabrication conditions, limitations for wire bonding and other limitations that affect the sensitivity of the system.

- INA125 is very sensitive and rapid amplifier for the electronic circuit. The values of the Wheatstone bridge is very important to gain enough, so it is observed that the values must be higher than 1.8 kΩ.

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APPENDICES

APPENDIX A. Comsol Supplementary
APPENDIX B. Fabrication Of Microfluidic Chip
APPENDIX C. Wire Bonding Parameters
APPENDIX D. Arduino Programming

APPENDICES

APPENDIX A. COMSOL SUPPLEMENTARY

APPENDIX A1. Geometry

The microfluidic chip consists of three main subdomains: Glass substrate, heating element and a protective layer on the heating element. A glass slide whose dimensions are 15 mm x 15 mm x 1 mm was used as substrate material and the slide was incorporated at x=9650, y= -1500, z=-1000 in microscale.

Heating element consists of a heater and an integrated temperature sensor which are made from Aluminum and the thickness of Aluminium coating is adjusted to 300 nm. The schematic of the heating element was uploaded by using workplane section, because the thickness of the Al coating is too low and it does not allow to mesh correctly and workplane section allows to upload the geometry at 2D.

The protective layer is made from SU8 which is a light-sensitive polymeric material and the dimensions of SU8 layer are 3000 μm x 3000 μm x 15 μm and the block is incorporated at x= y= in microscale.

APPENDIX A2. Properties of materials

Properties	Symbol	Glass	Aluminium	SU-8 3000
Heat capacity at constant pressure	Cp	703	904	1200
Thermal conductivity	k	1.38	237	0.2
Relative permittivity	epsilonr	3.78	1.6	3.2
Density	σ	2203	2700	1.153
Reference resistivity	ρ_0	-	2.65e-8	-
Resistivity temperature coefficient	α	0.55e-6	0.00429	-
Reference temperature	T	-	293.15	-

APPENDIX A3. Physics Modules

The 3D simulation of the system was performed with combination of Joule heating module which consists of AC/DC module and Heat Transfer module, and Shell,

Conductive Media, DC modules. The descriptions of the modules are adopted from the COMSOL's AC/DC Module User Guide.

A.3.1. AC/DC Module

AC/DC module serves many options to solve several problems in electromagnetic and the application modes of the module can be divided into two main fields: electrostatic fields, and magnetostatic and quasi-static fields. Two application mode which are the Conductive Media DC and Shell and Conductive Media DC are used in this simulation as a part of the electrostatic fields.

A.3.1.1. Conductive Media DC

The Conductive Media DC application mode solves the following partial differential equation (PDE):

$$Q_j = -\nabla . (\sigma \nabla V - J^e) \qquad \text{(A.1)}$$

where **Q***j* is the current source, σ is the electrical conductivity of the conductive media, *V* is the electrical potential, and **J***e* is an externally generated current density.

For the metals, the conductivity is temperature-dependent as described at equation A-2. The equation consists of ρ_0 is the resistivity at the reference temperature T_0, α is the temperature coefficient of resistivity (TCR), and T is the current temperature which is obtained from heat transfer module.

$$\sigma = \frac{1}{\rho_0[1+\alpha\,(T-T_0)}} \qquad \text{(A.2)}$$

The equation A.3 is demonstrated the following natural boundary condition to indicate the relevant interface condition at the interface between two different media (1 and 2).

$$n_2.(J_1 - J_2) = 0 \text{ (A.3)}$$

Where n is the normal vector and J is the externally generated current density at both media. Therefore, the boundary conditions typically adopted in this model with using equation A.4.

$$n.[\,(\sigma \nabla V - J^e)_1 - (\sigma \nabla V - J^e)_2] = -n.(J_1 - J_2) = 0 \text{ (A.4)}$$

A.3.1.2. Shell, Conductive Media DC

The *Shell, Conductive Media DC* application mode is used to model thin layers of conductive media by solving the problem on 2D surfaces in a 3D geometry. It is similar

with *Conductive Media DC* application mode, and the module is based on the following equation:

$$dsQ_j = -\nabla_t\, ds.(\sigma\nabla V - J^e) \qquad \text{(A.5)}$$

where *ds* is the thickness of the layer (or shell) and the operator represents the tangential derivative along the layer.

A.3.2. General Heat Transfer Module

The Heat Transfer Module offers to study on all the fundamental mechanisms in heat transfer, consists of conductive, convective and radiative heat transfer in different combinations (with both surface-to-surface and surface-to-ambient radiation). The *General Heat Transfer* is the main application mode within this module. COMSOL solves the conductive heat transfer analysis via using following PDE:

$$Q = \rho C\frac{\partial T}{\partial t} - \nabla\,(k\nabla T) \quad \text{(A.6)}$$

where Q is the heat source, ρ is the density, C is the specific heat capacity, T is the temperature, t is time and k is the thermal conductivity. For the steady-state simulation, only the second term which consists of thermal conductivity, k is applied and the first term containing ρ and C vanishes.

Table A.1. The boundary conditions for General Heat Transfer Application Mode

Description	Boundary Condition
Heat flux	$-\mathrm{n}.(k\nabla T) = q_0 + h(T_{inf} - T)$
Insulation or symmetry	$-\mathrm{n}.(k\nabla T) = 0$
Prescribed temperature	$T=T_0$
Continuity for interior boundary	$-\mathrm{n}_u.(k_u\nabla T_u) - \mathrm{n}_d.(k_d\nabla T_d) = 0$

where q_0 is the inward heat flux, h is the heat transfer coefficient and *Tinf* is the temperature of ambient environment. **n** is the normal vector, with the subscripts *u* and *d* denote the upside and downside of the boundary, respectively.

The thickness of the heating element is defined with The *Conductive Layers* feature, of the *General Heat Transfer* application mode, without the need to geometrically create a fine mesh for them. The feature promises to simplify the geometry and

significantly reduce the required number of mesh elements via no variations in temperature and in-plane heat flux exist along the direction of the thickness of the layer.

$$d_s\,\rho_s C_s\,\frac{\partial T}{\partial t} + \nabla_t\,(-d_s\,k_s \nabla_t T) = \; q_{\partial\Omega} - q_\Omega \quad \text{(A.7)}$$

$$q_{\partial\Omega} = \; q_0 + h\big(T_{inf} - T\big) \quad \text{(A.8)}$$

$$q_\Omega = -n\,(k\nabla T + \rho_s C_s T u) \quad \text{(A.9)}$$

where *ds* is the layer thickness, ρ_s is the layer density, *Cs* is the layer specific heat capacity, *ks* is the layer thermal conductivity, $q_{\partial\Omega}$ is the heat flux from the boundary into the layer and q_Ω is the heat flux from the layer into the sub-domain.

APPENDIX B. FABRICATION OF MICROFLUIDIC CHIP

APPENDIX B1.Heating element

Lift – off process

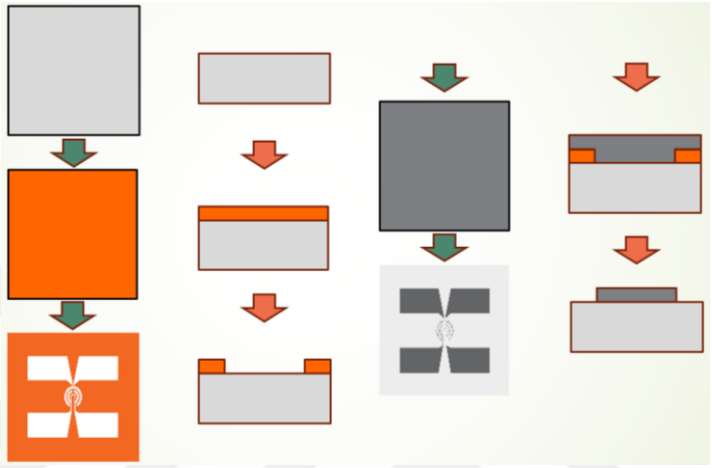

Figure B.1. The top views and cross-sections after each steps of lift-off process

Photoresist Spin Coating

- Spin coat AZ1505 photoresist for 10 seconds at 500 rpm by 100 rpm/sec acceleration and 40 seconds at 4000 rpm by 1000 rpm/sec acceleration.
- Soft Bake at 95°C for 50 seconds.

Photolithography

- Exposure time : 5 seconds
- Development parameters: 15 seconds in 1:4 AZ351B: DI Water (by volume) with gentle agitation followed by a 1 minutes rinse in gently running water.

Thermal Evaporation

For spiral boat:

- Current : 30 A
- Power : 32,5 %
- Deposition rate: 2.7-3.2 A^o

For rectangular boat:

- Current : 105 A

- Power : 35 %
- Deposition rate : 0.8-1 A^o

Lift was performed in acetone kept in an ultrasonic cleaner at room temperature for 30 minutes.

APPENDIX B2. Reaction Chamber

PDMS Mold Preparation

- Wafer is washed by acetone, isopropyl alcohol and water, respectively, dried with nitrogen gun and waited at 95°C for 10 minutes.

Photoresist Spin Coating

- Spin coat SU8 3050 photoresist for 10 seconds at 500 rpm by 100 rpm/sec acceleration and 35 seconds at 1000 rpm by 300 rpm/sec acceleration.
- Soft-bake at 95°C for 45 minutes

Photolithography

The geometry of the mold is transferred to the SU-8 layer by using photolithography. The exposure dose is given 250 mJ/cm2 for 100 μm thickness, so the photolithography is performed at 10 seconds.

- Post exposure bake at 65°C for 1 minutes and 95°C for 2-3 minutes.
- Development parameters:
  Developer: Dev600
  Development time: 15 minutes with gentle agitation and 1 minutes rinse with water.

PDMS Molding

- 5 g PDMS base is poured onto a clear container on the electronic weighing machine to adjust the amount of the curing agent in the ratio 10 :1
- Carefully 0.5 g the curing agent is added onto PDMS base.
- The mixture is whisked vigorously with a spatula until the mixture is filled with air bubbles for about 10 mins. Thorough mixing, the curing agent must be uniformly distributed to ensure that the final PDMS mold is uniformly cross linked.
- The mixture is placed in a dessicator which is connected to a vacuum pump for trapping air bubbles escape for about half hour.

- After obtaining a clear, bubble free PDMS mixture, it is poured onto SU-8 molds have been used.
- PDMS with mold is cured on a hotplate at 100^{o}C for 35 min.

APPENDIX C.WIRE BONDING PARAMETERS

Aluminium wedge bonding process is performed with using the parameters which are demonstrated at Table-B.2 . 33 μm Al wire is used and the process is performed at room temperature.

Table C.1. The parameters for Aluminium Wedge Bonding

Ultrasonic Force - 1	490	Time - 1	200	Force - 1	325
Ultrasonic Force - 2	600	Time - 2	200	Force - 2	400

APPENDIX D.ARDUINO PROGRAMMING

```
void setup() {
  Serial.begin(9600);
  Serial.println("CLEARDATA");
  Serial.println("LABEL,Time,voltage2");
}
void loop() {
  Serial.print("DATA,TIME,");
  delay(100);
  int switcha= 3;
  int sensorDegeri1 = analogRead(A0); /* Arduinonun A0 ayagindaki gerilim
olculuyor */
  pinMode(switcha,OUTPUT);
  float voltage1= sensorDegeri1*5.00;
  float voltage2= voltage1/1024.000;
  //Serial.print("heater=");
 if ( voltage2 > 2.7) {
digitalWrite(switcha,LOW);
  }else {
  digitalWrite(switcha,HIGH);
  }
  Serial.println(voltage2,4);
  delay(100); /* daha dogru bir olcum icin biraz bekleme kullanilmalidir */
 //  Serial.println(voltage2);
 // delay(1000);
}
```

8. CURRICULUM VITAE

Name Surname **: ZEYNEP BURCU ÇAVDAR**

Place and Date of Birth **: 31.05.1990 / KARTAL**

E-Mail **: cavdarzey@itu.edu.tr**

EDUCATION **:**

- **B.Sc.** **:** 2015, Istanbul Technical University, Faculty of Chemical and Metallurgy , Chemical Engineering

PROFESSIONAL EXPERIENCE AND REWARDS:

- 2014-2015 B.Sc. Thesis *"Carbon Materials as Radar Absorbing Materials"*
- 2015 Scholarship from BAP for the project *" Production and Characterization of Polyacrylonitrile(PAN) based activated carbon nanofibers "*
- 2015-2017 Scholarship from TUBITAK for the project "*Design, production and integration of LAMP based microfluidic chip for diagnosis of tuberculosis"*

PUBLICATIONS, PRESENTATIONS AND PATENTS ON THE THESIS:

OTHER PUBLICATIONS, PRESENTATIONS AND PATENTS:

Pelin ALTAY, Zeynep Burcu CAVDAR, Nuray UCAR, Nilgun KARATEPE YAVUZ (2015). Analyses of Carbon and Activated Carbon Nanofiber Web , Proc. of The Third Intl. Conf. On Advances in Applied Science and Environmental Technology - ASET.

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