

**Microcantilever Based Lab-on-a-Chip Sensor for Real-Time
Mass, Viscosity, Density and Coagulation Measurements**

by

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ABSTRACT

Miniaturization of sensor technologies and the development of lab-on-a-chip (LoC) devices in the last decade enabled new opportunities in diagnostics and therapeutics to improve human health. Despite the great technological advances in research labs, there are only a few commercially successful LoC devices used for point-of-care diagnostics applications. Such applications demand simple sensor readout and single-use disposable sensors, which are difficult requirements to achieve due to mechanical, fluidic, optical, and electronic interface and integration problems. This thesis focuses on the development of a fully integrated LoC measurement system that use micro-electro-mechanical-system (MEMS) based microcantilevers operated in a microfluidic cartridge with simple interface and simple sensor readout.

Microcantilever biosensors developed in this research have been adapted for different point-of care applications (blood plasma viscosity and coagulation time measurements), liquid property monitoring and immunoassay detection purposes. The cantilevers are made of electroplated Nickel and fabricated with a simple process using Koç University Micro/Nano Fabrication Clean Room. Cantilever designs varied according to the application. The length of the cantilevers are between 60 μm and 200 μm , the width varies between 5 μm and 100 μm and the thickness is generally in the order 1- 2 μm . They are fully immersed in liquid and actuated by an external electro-coil that operates close to cantilever resonant frequencies and sensing is conducted by means of an optical read-out. A unique optical interferometric method or a laser Doppler Vibrometer is employed for the optical read-out. Both actuation and read-out are conducted remotely. Thus electrical connections to the cantilevers are not required. This feature enables the use of the developed technology as a point of care device with disposable cartridges. The oscillation

dynamics of the cantilevers change due to the variations in the liquid properties or the accreted mass on the cantilever surface. The changes in the dynamics are either tracked with lock-in amplifier or phase-locked loop (PLL) based electronics that follows the optical detector. The lock-in amplifier method tracks the phase difference between the actuation signal (coil drive current) and the sensor signal (photo detector output) whereas the PLL method tracks the oscillation frequency real-time by using a fixed phase difference between the actuation and the sensor signals.

The cantilever biosensor platform has been used for four biological applications during this thesis research. First, the system is utilized as a viscosity sensor and viscosities blood plasma and blood serum are measured. Blood plasma viscosity can be used for the diagnosis of several diseases such as cardiovascular disorders, rheumatoid arthritis, and certain autoimmune diseases. The proposed system enables fast and convenient measurements with small sample volumes ($\sim 10\mu\text{l}$), which is superior to common bench-top clinical devices and makes the system suitable for point of care use. New protocols are developed, the systems repeatability and reliability is also tested with reference measurements using commercial laboratory devices. For viscosity detection a detection limit of $0.01\text{ mPa}\cdot\text{s}$ is achieved for blood plasma with error less than 6%. Second, a novel method is proposed and demonstrated to separate the coupled effects of density and viscosity on the cantilever oscillation dynamics. A set of equations and a simple algorithm is developed to relate the density and the viscosity to the frequency shifts of the cantilevers. We found that the effect of the density and the viscosity can be well separated if cantilevers have different widths. The method uses the PLL based system to track the resonant frequencies of two cantilevers with different widths immersed in the same liquid. Precise density and viscosity measurements are performed and compared with the reference measurements. The measurement error with the new method was lower than 3 % in density in the range $995\text{ to }1150\text{ kg/m}^3$ and 4.6 % in viscosity in the range $0.935\text{ to }4\text{ mPa}\cdot\text{s}$. Based

on the signal-to-noise ratio, the minimum detectable difference in the viscosity is 1.6×10^{-3} mPa.s and the density is 0.3 kg/m^3 .

As the third application, the system is adapted for blood plasma coagulation time tests. Periodical coagulation time tests are required for patients who are receiving anticoagulant therapy, undergoing pre-operation evaluation or for patients under risk of embolism, stroke or atrial fibrillation. In general practice, patients need to visit a hospital or a central laboratory periodically for coagulation tests. Such a procedure puts a significant burden on the health-care provider and increases the return time and the cost of the test. Thus, fast, reliable and simple assays are needed to monitor the coagulation parameters. For this purpose the MEMS chips containing cantilevers are integrated with microfluidic channels and multiplexed coagulation time measurements are enabled from single cartridge. New protocols are developed for standard coagulation tests (Prothrombin time (PT) and activated partial thromboplastin time (aPTT)). The tests are conducted not only with standard control plasma samples but also with human plasma samples. The measurement system has an overall 7.28 % and 6.33 % CV (Coefficient of Variation) for PT and aPTT, respectively which are comparable with the commercial devices with different technologies. As the fourth application, the system is tested for immunoassay detection in parallel channels. The proteins of Hepatitis B, Hepatitis C, HIV and Syphilis antigens are detected with reasonable detection limits.

The technology developed in this thesis is simple to use, label-free, can be integrated in a disposable cartridge, and suitable for multiple tests in the same cartridge; therefore, it has high potential for point-of-care and home diagnostics applications.

Keywords: MEMS, BioMEMS, cantilever, Point-of-Care test, Lab-on-a-Chip, microfluidics, cartridge, viscosity, density, blood, blood plasma, coagulation time, PT, aPTT, antigen detection

ÖZET

Son yıllarda gerek sensör boyutlarındaki küçülme gerekse “lab-on-a-chip (LoC) araçlarındaki gelişmeler insan sağlığını olumlu yönde etkileyecek teşhis ve tedavi yöntemlerine imkan tanımıştır. Araştırma laboratuvarlarındaki bu gelişmelere rağmen bu tip teknolojilerin ticarileştirilmesi ve yaygın bir kullanım kazanması kısıtlı kalmaktadır. Bu tip uygulamalar için basit algılama arayüzleri ve tek kullanımlık sensörler gerekmektedir. Bu zor gereklilikler ancak mekanik, akışkan, optik ve elektronik arayüzlerin iyi bir şekilde entegre edilmesiyle aşılabilmektedir. Bu tez çalışmasında tam entegre bir LoC ölçüm sistemi geliştirilmiştir. Bu sistemde sensor teknolojisi olarak mikro-akışkan kartuş içerisinde çalışan, basit bir arayüz ve algılama teknolojisine sahip mikro-elektro-mekanik-sistem (MEMS) tabanlı mikroçubuklar kullanılmıştır.

Bu tez çalışması kapsamında geliştirilen mikroçubuk tabanlı biyosensor teknolojisi farklı medikal görüntüleme uygulamaları(kan plazması viskozitesi ve pıhtılaşma zamanı ölçümleri), akışkan özellik ölçümleri ve virüs tespiti amaçlarıyla kullanılmıştır. Mikroçubuklar Koç Üniversitesi Mikro/Nano İmalat Temiz Odası’nda imal edilmiş olup, elektro kaplama metoduyla üretilen Nikel malzemeden yapılmıştır. Mikroçubuk tasarımları farklı uygulamalar için modifiye edilmiştir. Çubuk uzunlukları 60 μm ile 200 μm arasında genişlikleri 5 μm ile 100 μm arasında değişmekte olup, kalınlıkları da 1-2 μm mertebesinde. Tamamen sıvıya daldırılmış olup elektro-bobin eyleyici yardımıyla rezonans frekansları civarında tahrik edilmektedir. Ayrıca çubuk titreşimleri de farklı optik okuma düzenekleri ile algılanmaktadır. Çalışma sırasında özel geliştirilmiş girişimsel (interferometric) okuma metodu veya lazer Doppler Vibrometre tabanlı okuma kullanılmıştır. Hem tahrik hem de algılama temassız olarak gerçekleşmektedir. Böylelikle mikroçubuğa herhangi bir elektriksel bağlantıya ihtiyaç duyulmamaktadır. Bu özellik sayesinde geliştirilen teknoloji tek kullanımlık kartuşlara sahip hasta başı testlere (point-of-

care) dönüştürülebilmektedir. Mikroçubuk dinamiği içerisinde salınım yaptığı akışkanın özelliklerine göre veya mikroçubuğa bağlanan biyolojik kütlelere bağlı olarak değişmektedir. Bu değişimler “lock-in yükselteç” veya “Phase-Locked Loop (PLL)” yardımıyla görüntülenebilmektedir. Lock-in yükselteç sabit bir tahrik frekansında tahrik sinyali ile optik okuma sinyali arasındaki faz farkını ölçmekteyken, PLL ise bu faz farkını sabit tutup rezonans frekansındaki değişimleri anlık olarak takip edebilmektedir.

Mikroçubuk tabanlı sensör platformu dört ana uygulama için kullanılmıştır. İlk olarak, sistemde kan plazması ve kan serumu akışkanlığı ölçülmüştür. Kan plazması viskozite ölçümleri kardiyovasküler bozukluklar, romatizmal kireçlenme ve çeşitli otoimmün hastalıkların teşhisinde kullanılmaktadır. Önerilen sistem çok küçük numuneler ile (~10µl) hızlı ve güvenilir ölçümler yapabilmekte olup bu özellik mevcut laboratuvar cihazlarına göre büyük bir avantaj sağlamaktadır. Bu ölçümler için yeni ölçüm protokolleri oluşturulmuş ve sistemin tekrarlanabilirliği ve güvenilirliği referans ticari cihazlarla kıyaslanarak test edilmiştir. En küçük ölçülebilen kan plazması viskozite değeri 0.01 mPa.s olup ölçüm doğruluğu %6’dır. İkinci olarak, özkütle ve viskozitenin mikroçubuk dinamiği üzerindeki akupole etkilerini ayırtabilmek için yeni bir metot geliştirilmiştir. Basit bir algoritma ile viskozite ve özkütle değerlerinin rezonans frekansı üzerine olan etkileri belirlenmiştir. Böylelikle farklı genişlikteki iki adet mikroçubuk kullanılarak bu etkilerin birbirinden ayrıştırılabileceği gösterilmiştir. Sistem ile alınan hassas ölçümler referans cihazlar ile karşılaştırılmıştır. Kullanılan metot ile 995 kg/m³ ile 1150 kg/m³ arasındaki özkütle ölçümlerinde %3 doğruluk, 0.935 mPa.s ile 4 mPa.s arasındaki viskozite ölçümlerinde %4,6 doğruluk elde edilmiştir. Ayrıca ölçülebilecek en küçük viskozite değeri 1.6x10⁻³ mPa.s ve özkütle değeri 0,3 kg/m³ dir.

Üçüncü uygulama olarak, sistem kan plazması pıhtılaşma testleri için adapte edilmiştir. Sürekli antikoagulan ilaç kullanan hastalarda, ameliyata girecek hastaların değerlendirilmesinde ve emboli riski taşıyan hastalarda periyodik olarak pıhtılaşma

sürelerinin ölçülmesi gerekmektedir. Bu amaçla, hastaların sürekli olarak kliniklere gitmeleri ve bu testleri yaptırmaları gerekmektedir. Bu prosedür sağlık sistemleri üzerinde önemli bir yük olup test sürelerinin uzamasına neden olmaktadır. Bu sebeple pıhtılaşma ölçümleri için hızlı, güvenilir ve basit sistemlere ihtiyaç duyulmaktadır. Bu amaçla, MEMS çipler mikroakışkan kanallarla entegre edilmiş ve tek bir kartuştan çoklu pıhtılaşma zamanı ölçümleri gerçekleştirilmiştir. Protrombin Zamanı (PT) ve aktive Parsiyel Tromboplastin Zamanı (aPTT) ölçümleri için yeni protokoller geliştirilmiştir. Testler hem standart kontrol plazmaları ile hem de gerçek insan plazmaları ile yapılmıştır. Sistem PT ve aPTT ölçümleri için sırasıyla %7.28 ve %6.33, %CV(coefficient of variation) tekrarlanabilirlik değerlerine sahip olup bu değerler mevcut cihazlarla kıyaslanabilecek seviyededir. Dördüncü uygulama olarak da sistem çeşitli antijenlerin tespiti amaçlı kullanılmıştır. Hepatit B, Hepatit C, HIV ve sifilis virüslerinin proteinleri iyi hassasiyetle ölçülebilmektedir.

Bu tez çalışması kapsamında geliştirilen entegre teknoloji yardımıyla tek kullanımlık kartuşlarla birden fazla testin kolayca ve etiketleme gerektirmeden yapılması mümkün olmuştur. Dolayısıyla çalışma, hasta başı ve ev içi tanı ve görüntüleme test uygulamaları için yüksek bir potansiyel taşımaktadır.

Anahtar Kelimeler: MEMS, BiyoMEMS, mikroçubuk, Hasta Başı Test, mikroakışkan kartuş, viskozite, özkütle, kan, kan plazması, pıhtılaşma zamanı, PT, aPTT, antijen, teşhis, tanı

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1 INTRODUCTION

In this chapter, measurement principles of biosensors and their classification are discussed. A literature review of micro/nano mechanical sensors, covering their main types, operation modes and sensing and actuation principles are presented. The achievements of different groups using different techniques are reviewed. Afterwards the state of the art in viscosity and density sensing, coagulation time measurements and biological mass sensing is underlined. Then the contributions of this thesis are briefly presented.

1.1 Biosensors

A biosensor is an analytical device, used for the detection of an analyte that combines a biological component with a physicochemical detector [1]. In the last few decades, especially after the discovery of DNA chain structures there have been a boom in the biosensor research. Clark, Lyons and Hicks developed the first biosensor in 1960s[2, 3]. Their method was based on monitoring the specific catalytic interaction of the glucose oxidase enzyme and glucose. For many vital diseases, like cancer, the early and convenient diagnosis plays a key role for the treatment. Also there are many other diseases, like diabetes and cardiovascular problems, whose vital symptoms need to be kept under control with well-adjusted medication. In order to provide the critical dose adjustments, frequent testing and monitoring is required. Viral epidemics like EBOLA, SARS, H1N1, HIV, and Hepatitis caused many deaths in the last decade and are still threatening the public health all over the world. Fast and reliable biosensors, which are able to detect even very small concentrations from a small sample volume, are needed for early diagnostics of contagious diseases. Point-of-care and home-care diagnostics systems provide easy access to the

healthcare system and increase the quality of life particularly for resource-limited settings while decreasing the burden on the economy.

The general working principle of a biosensor is shown Figure 1.1. A surface is immobilized with a receptive layer having a biological affinity with the target molecules. If the target molecules like antigens, proteins, enzymes or other types of analytes exist in the examined sample they attach to the immobilized surface. This attachment induces a signal. This signal is transduced to another domain, such as optical, mechanical, electrical which can be measured in a quantitative way. Some amplification may be necessary during these steps. Also the physical properties of a biological liquid such as viscosity and density can be monitored by using biosensors.

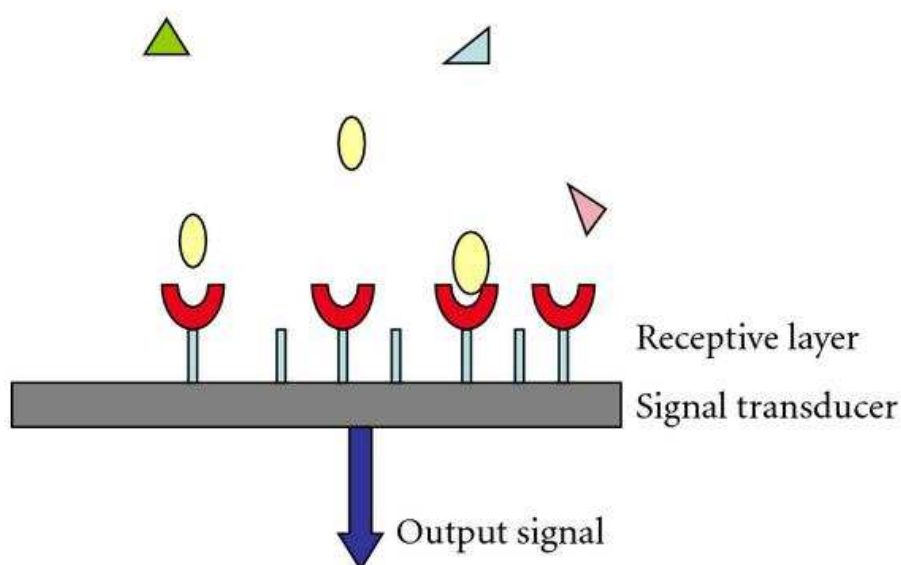


Figure 1.1: Principle of a biosensor construction[4].

Labeled and Label-Free Biosensors: In different types of biosensors a label or marker is attached to a secondary probe molecule to enable readout (for instance, fluorescent assays) or to amplify the signal (for example, labelling with relatively massive

nanoparticles for mass-based detection). This operation is called **labeling** and it requires sample preparation steps and increase the test duration. One of the most common and mature technology used in the clinics is enzyme-linked immune assays (ELISA) tests. Generally sandwich assays are used with the ELISA tests. In sandwich assays two affinity-based probes (often two different antibodies) are utilized to attain an effective affinity. This is a labelling operation. The main drawback of the ELISA tests is their long response time which is related to this labelling operation. However with the **label-free** biosensors it is easier to miniaturize the system and reduce the complexity of the process, which avoids the laboratory requirement. This also significantly affects the response time. The microcantilever based sensor developed in this thesis is a label-free biosensor.

The main metrics for the biosensors are linearity, sensitivity, selectivity, response time and sample volume. Many different technologies are competing with each other to achieve high performance in these metrics. Figure 1.2 shows the classification of the biosensors depending on the physical domain of the transducer. They can be classified under three main categories, electrical, optical and mechanical [5]. Optical biosensors are very common and there has been intensive research on them. Some of the frequently used biosensors are subdivisions of this type. Lateral Flow Assays (LFA) (pregnancy tests) and ELISA is colorimetric biosensors. Surface Plasmon Resonance (SPR), fluorescence based sensors are also under this category. Electrical biosensors also have a common usage. Most of the commercial glucose monitoring devices are electrochemical sensors. Also another cutting edge technology, nanowires [6-8] can be classified under electrical biosensors.

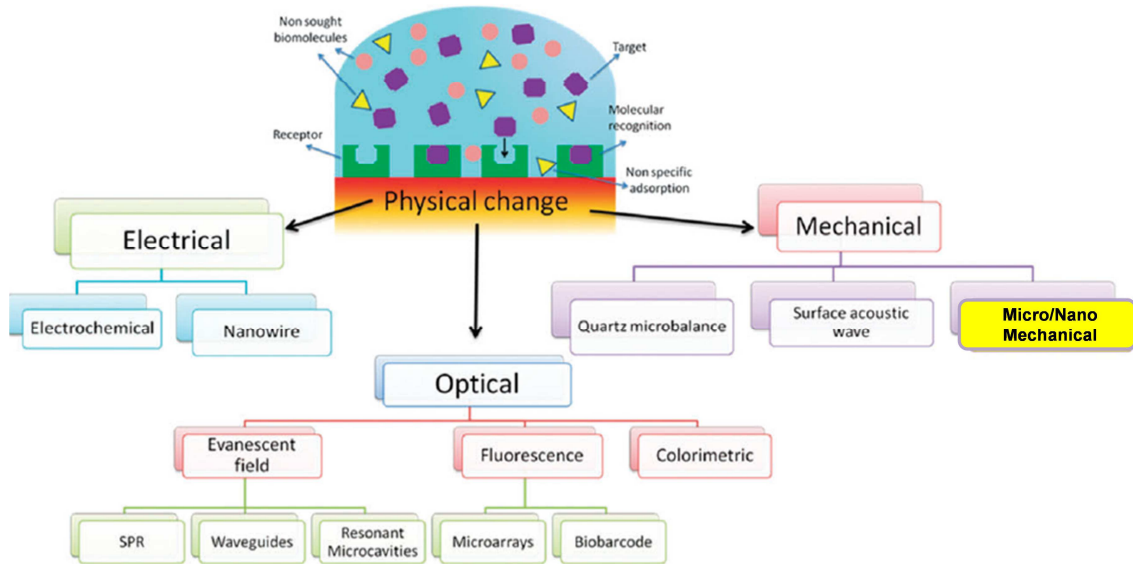


Figure 1.2 Schematics of a biosensor and biosensor subclasses. Biosensors can be divided into electrical, optical and mechanical biosensors, depending on the kind of physical change. Each category includes different types of biosensor devices. [5]

In the mechanical biosensors category Quartz Crystal Microbalance (QCM) [9-12] and surface acoustic wave based technologies are mature and commonly used techniques. The cantilever-based sensors, which are used in this thesis, are micro/nano mechanical sensors. The ability to fabricate micro and nano scale devices with wafer-scale semiconductor processes has emerged an attention for the development of practical micro- and nanoelectromechanical biosensors that can be produced with mass production [13]. First, nanoscale mechanical sensors provide high mass resolution since the target mass and the mass of the device is comparable. Second, with the aim of the matured transducing techniques the mechanical response of the device to biological and chemical reactions can be converted into electrical signals easily. Finally with the growing research in the

microfluidics field small and robust fluidic devices have been developed which enables fast response times. There is high potential to use the micro/nano mechanical sensors as sensitive point-of-care and diagnostic devices by integrating them with microfluidics. There are two important metrics that should be considered while developing a new biosensor, analysis time and limit of detection [13]. Roukes group claims that the newly developed biosensors should be able to compete with the standard test tools as LFA and ELISA [13].

Before discussing the details of cantilever sensors, point-of-care testing and Lab-on-a-Chip concept will be introduced briefly.

1.1.1 Point-of-Care Testing

Point-of-Care testing is an application area of biosensors which enables medical testing near the site of patient care. This type of testing is mainly used by the patients having chronic diseases (diabetes and cardiovascular problems) and for the monitoring of critical conditions, like pregnancy. These devices are easy to use and the tests can be conducted by the patient or clinical technicians. They generally require a small sample of a body liquid (blood or urine) [14] and respond in a short time [15]. For instance, the diabetes patients have to use medication and enzyme supplement to control their glucose level. The dosage of the medication is critically adjusted depending on the daily or weekly measured glucose levels. By glucose level monitoring devices the patient can conduct these measurements without going to clinics and prevent from potential risks. This concept is also applicable for cardiovascular patients with a risk of embolism. Their coagulation times should be measured periodically and the dosage of the anti-coagulant should be adjusted [16]. Fast diagnosis of critical diseases, like HIV can also be detected by point-of-care devices [17]. Especially in the underdeveloped countries threatened by the epidemics, these kind of practical devices are vital since the clinics are not easily accessible by the society. The point-of-care concept covers not only the hand-held devices which can be operated by the

patient but also the bench-top devices which are used in the clinics enabling fast diagnostics and monitoring. Some commercial Point-of-Care devices are shown in Figure 1.3.

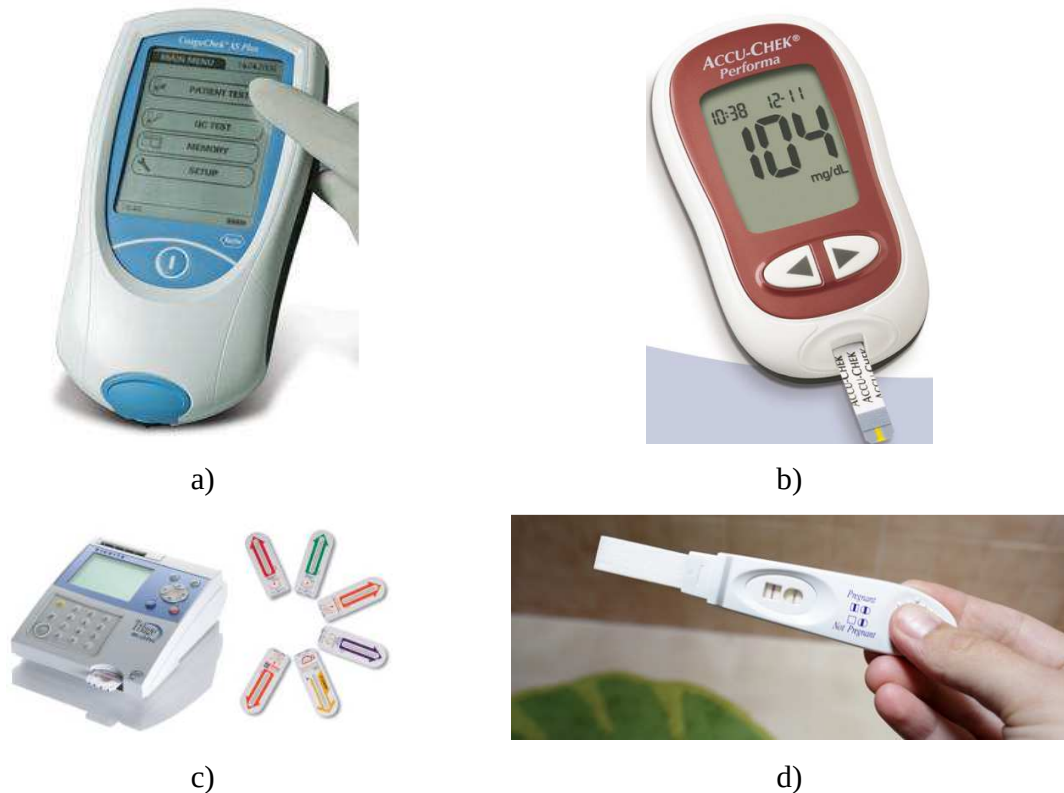


Figure 1.3: Some Point of Care devices. a) Roche Coagucheck for Prothrombin Time (PT) Tests [18]. b) Roche Accucheck for glucose level monitoring [14]. c) Alere Triage for rapid diagnosis of critical diseases, like HIV [17]. d) Pregnancy test from urine [15].

Another concept named as Lab-on-a-Chip (LoC) which utilizes microfluidic channels also enabled diagnostics with small sample volumes and low concentrations [19]. This concept enabled very fruitful biological research from single cell analysis [20, 21] to drug discovery [22]. The microfluidic channels can be made of low-cost material, require low

sample volumes and combined with the MEMS sensor technology thus allow disposable cartridges for point-of-care devices.

1.2 Cantilever Based Sensors

The cantilever sensors are robust and mature technologies with generally simple fabrication. They can be fabricated in different scales from nanoscale to millimeter scale. Atomic Force Microscopes (AFM) mainly opened the era of cantilever-like sensors and they have been used for different applications from gas sensing to biosensors. As will be discussed in details in the upcoming sections, their use for biological agent detection in liquid environment is limited due to viscous damping effects. Nevertheless they are very convenient tools for liquid property monitoring. Since this type of sensors is the focus of this thesis, the details of sensing modes, actuation and sensing principles and state of the art will be covered with details in sections 1.2.1 and 1.2.3. The theoretical background and the measurement principle will be covered in sections 2.1 and 2.3.

1.2.1 Sensing Modes of Cantilever-Based Sensors

The microcantilever based biological and chemical sensors operate in two different modes, static and dynamic modes as shown in Figure 1.4. In the static mode the static bending due to affinity binding is measured whereas in the dynamic mode the resonance frequency shift due to the added mass is measured.

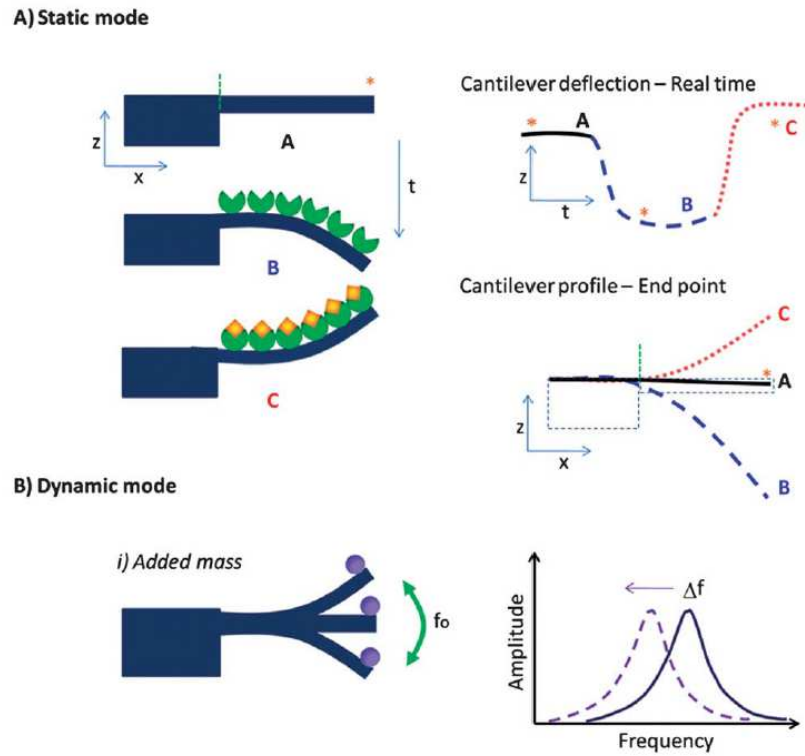


Figure 1.4: a) Static-mode cantilever. b) Dynamic mode cantilever [23].

1.2.1.1 Static Mode Cantilevers

In the first mode of operation, static deflection of the cantilever is monitored. Static deflection of a cantilever changes due to the difference in surface stress of the two faces of the cantilever (Figure 1.5). One of the surfaces is functionalized for specific binding. The binding of the biological or chemical agents results in a stress variation between the up and down layer. The early use of this method was reported for the detection of prostate-specific antigen (PSA) by Thundat et al. [24], DNA detection by IBM Zurich groups [25], protein detection by Manalis group [26], microorganisms by Kasas group at EPFL [27] and

recently for the monitoring of antibiotic susceptibility of bacteria by Gerber group at University of Basel [28]. However, the low signal-to-noise ratio observed during detection limits the success of this method. Biomolecular detection has typically generated small surface stresses ($\sim 0.001\text{--}0.01\text{ N m}^{-1}$) which is not easily measurable [29]. Also they cannot be used for liquid property sensing applications.

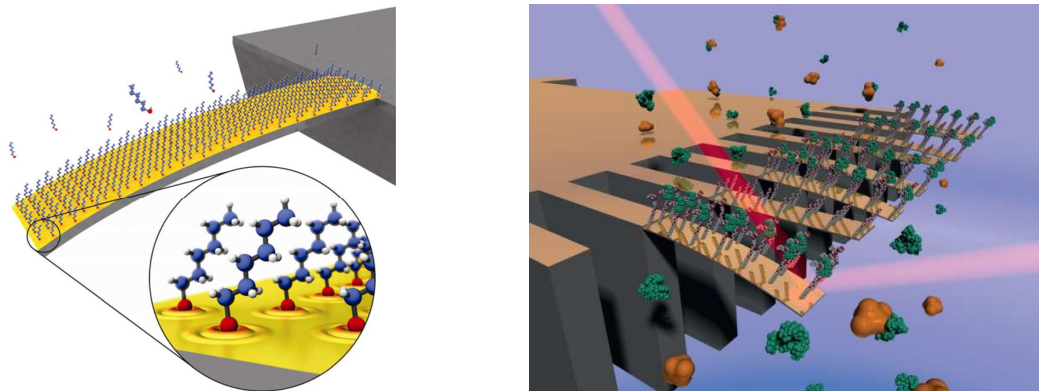


Figure 1.5: Static-mode cantilever sensors [28, 29]. The targeted agents bind to functionalized surface and create a stress and bending.

1.2.1.2 *Dynamic Mode Cantilevers*

The second operation mode is the dynamic mode, where the dynamic behavior of the cantilever changes according to the binding or liquid media property variations. In this thesis this mode of operation is utilized for specific applications. As shown in Figure 1.4 when binding occurs the resonant frequency of the cantilevers is shifted due to the added mass. This shift can be monitored to determine the amount of bonded agent. Researchers have used this mode for sensing in mainly three different environmental conditions [13]: The first one is a very common and mature way; the dip and dry technique. The cantilevers resonance frequency is measured prior to the binding in air or in vacuum. Afterwards the sensor is dipped into the solution containing the target agents and the incubation occurs for a certain time depending on the analytes. After the binding the cantilever sensor is dried

generally with a critical drying compatible with the bonded agent. Finally the resonance frequency in air or in vacuum after desiccation measured again and the amount of binding is determined from the frequency shift. Craighead Group at Cornell University and Bashir Group at Purdue showed the early efforts in this area on the detection of relatively massive bacteria cells [30] and achieved single-virus resolution [31], respectively. Craighead and coworkers also used ‘sandwich assay’ to detect prostate-specific antigen (PSA) in serum at femtomolar concentrations [32]. However, in this technique large molecules can bind non-specifically to the cantilever during desiccation, which leads to significant errors [13]. Also dip and dry is not applicable to liquid property sensing.

The second one is a recently emerged application area of dynamic-mode cantilevers which is to real-time monitor the bacterial and fungi growth in humid environments [33-35].

The continuous monitoring of the cantilever response in liquid environment is the third approach. The method is advantageous compared to dip and dry since it enables real-time and fast monitoring. However due to the viscous damping in liquid environment the Quality Factors (Q) are low which results in a low Signal-to-Noise Ratio (SNR) and low sensitivity compared to dip and dry technique[36]. These limitations will be treated in details in the theory part in section 2.1. Only a few groups achieved to obtain biological mass detection using the dynamic mode cantilevers in liquid. Mutharasan group at Drexel University uses millimeter scale piezo cantilevers (Figure 1.6a) for biological detection, generally different type of bacteria (up to 10 cells/ml) and BSA proteins (up to 1 pg/mL~20 fM) [37-39]. They facilitated longitudinal modes to obtain high Quality Factors (around 25). Hegner group at University of Basel conducted research on in-liquid operation for virus detection (T5 virus). They achieved to measure up to 1.5 pM concentration by exciting the cantilevers at their 10th to 13th modes at high resonance frequencies, up to 1Mhz and high Q, up to 30 (Figure 1.6b). T5 virus protein have high molecular weight

(70000 kDa), thus it was possible to attain such low concentrations. The minimum mass detected corresponds to 4 ng in their study. Also they facilitated usage of reference cantilevers to avoid the drifts and frequency instabilities [40-42]. Our group also utilized microcantilevers operating in liquid media to detect hepatitis A and C antigens, in the earlier studies [43, 44]. The quality factors achieved were less than 4, nevertheless the reported minimum concentration was 0.1ng/ml which corresponds to 1.66 pM and 1ng total mass in 1 ml solution. For the case when cantilever is fully coated with one layer of antigen protein the detected mass value corresponds to 2 pg, which is the lowest level achieved with the cantilever sensors operating in liquid [5].

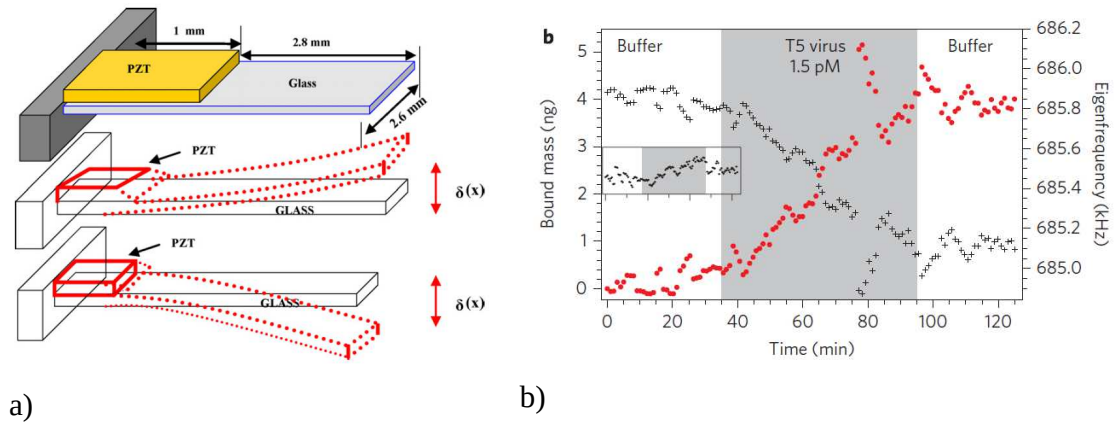


Figure 1.6: Dynamic mode cantilever sensors a) The cantilever sensor of Mutharasan Group at Drexel University [45]. b) A result with silicon cantilevers operated in buffer [40].

It is more convenient to use the dynamic cantilevers in liquid media to measure the properties of the liquid. The resonance frequency and the damping are highly related with the medium in which the cantilever operates. The liquid layer on top of the cantilever is accelerated with the cantilevers' motion. Hence this creates an “added mass” which results in a change in the resonance frequency of the cantilever. Also due to the viscous damping

the Quality factor variations occur depending on the viscosity of the liquid. These relations are expressed in analytical form by Sader in 1998 [36]. The theory will be treated in details in section 2.1.2. It is obvious that by tracking the frequency and quality factor viscosity and density of the liquid can be determined. Since the viscosity and the density effect is higher than a bonded biological analyte, lower quality factors and low SNRs are still satisfactory for this type of measurements. Dufour group at University of Bordeaux [46-54] and Jakoby Group at Vienna University of Technology [51, 55-58] are two main groups conducting research in this field. The main focus of this thesis is the viscosity and density sensing with dynamic mode cantilevers and its applications to biological fluids and Point of Care systems. Hence the literature related to viscosity and density sensing will be treated in details in section 3.1 and section 4.1.

The standard cantilever-based sensors have limited mass detection capability due to their low Quality Factors (Q) in liquid environment. To overcome this issue Manalis group at Massachusetts Institute of Technology (MIT) developed an intelligent solution in which instead of immersing dynamic mass sensors in the sample liquid they constrain the fluid to channels embedded in the cantilever resonator itself [59]. These Suspended Microchannel Resonators (SMRs) can be measured in vacuum and achieve Q values up to $\sim 15,000$. This opened a new area of research and today many different groups are working on enhancing the performance of SMRs. A disadvantage of this type is the microfabrication complexity. Despite the high values of Q, the early results of limit of detection of SMR biosensors were modest. They measured glycoprotein ALCAM in a serum dilution at 300 pM concentrations in several minutes [60]. SMRs have been applied to measurements of cell mass and density of yeast [61], and have been utilized for measuring growth rates from single cells of mammals [62]. Arlett et. al. have analyzed the ultimate practical limits of SMR sensors and calculated as 1.5 pM for proteins in 10 minutes [63]. Also a very recent

study of Manalis group shows that the method is capable of measuring mass in attogram levels.

Another area of use for SMRs is viscosity and density sensing [64, 65]. Boisen group at Technical University of Denmark were able to measure density and viscosity with an accuracy of 0.1 kg/m^3 and 0.025 mPa.s , respectively.

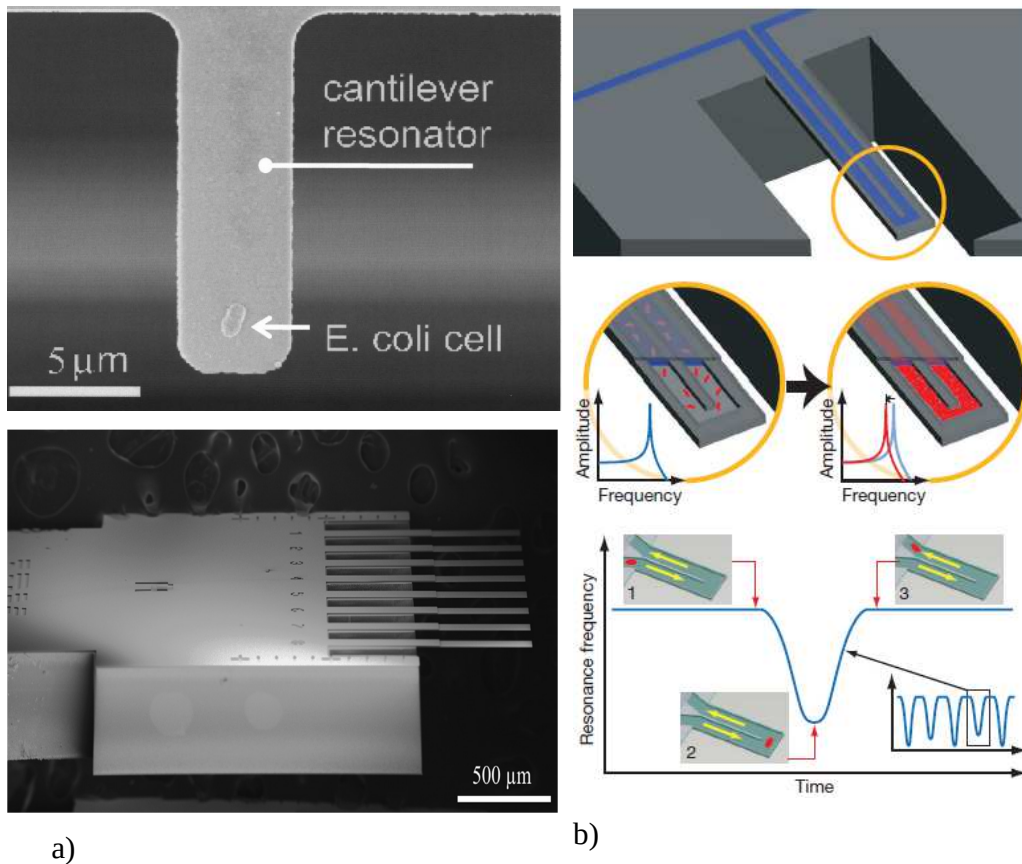


Figure 1.7: Micro/Nano Mechanical Biosensors. a) Cantilever Based Biosensors[30, 40].
b) Suspended Microchannel Resonators[59].

1.2.2 Actuation and Read-out Methods for Cantilever-based Sensors

Cantilever sensor based detection relies on real-time measurements of cantilever deflections at nanometer accuracy or better [66]. For the dynamic mode cantilevers there are various actuation methods to drive the cantilever. Different types of physics and materials have also been utilized to conduct the read-out. The most common and recent methods are listed in Figure 1.8. They will be briefly described and the literature using these methods will be covered in details.

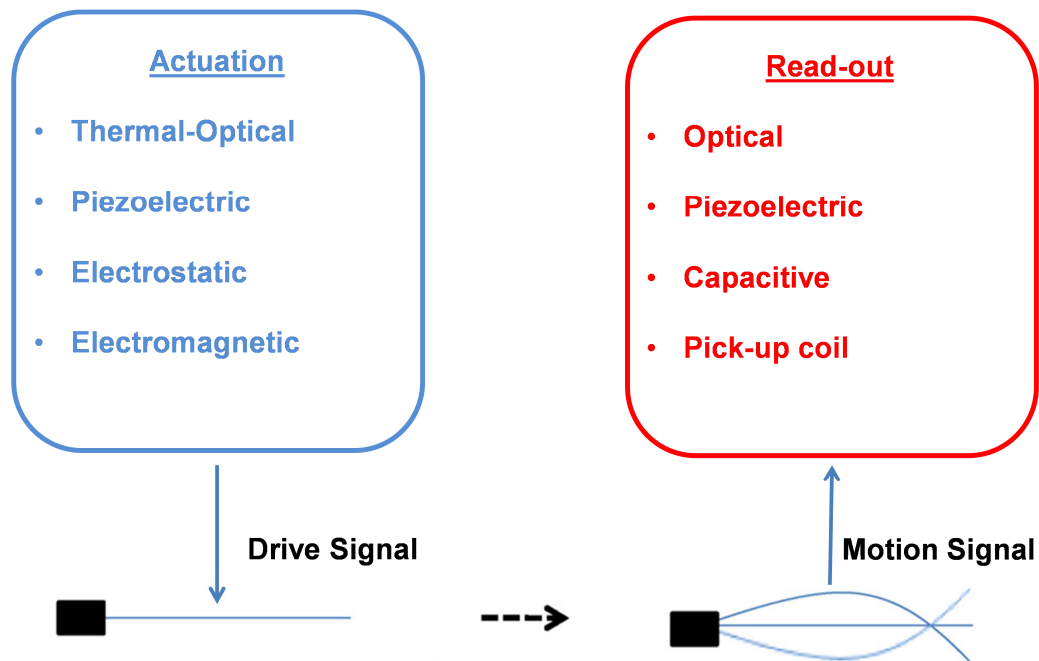


Figure 1.8: Excitation and Read-out methods in dynamic-mode cantilevers

1.2.2.1 Actuation Methods

Thermal-Optical: One easy way to actuate cantilever using thermal noise which is due to the Brownian motion of the molecules. With this approach the cantilever is only driven by the thermal noise, without any external actuation. The Craighead group in Cornell University achieved to detect single *Escherichia coli* (E. coli) cell using the thermal

actuation[30]. This study is one of the first in the literature employing cantilevers for biological mass detection. They dipped the cantilever inside the bacteria solution, dried the cantilever and measured the shift in the frequency. Also Bashir group at in Purdue University [31] detected a virus in *Poxviridae* family and Bascillus bacteria spores [67] again by measuring the frequency shift in thermal noise peak. However at room temperature, the thermal energy is very low and long measurement times are the consequence. The signal is very noisy (Figure 1.9a) and it is challenging to detect the frequency changes if the cantilevers are operating in a liquid medium unless the system has a high resonance frequency in MHz region.

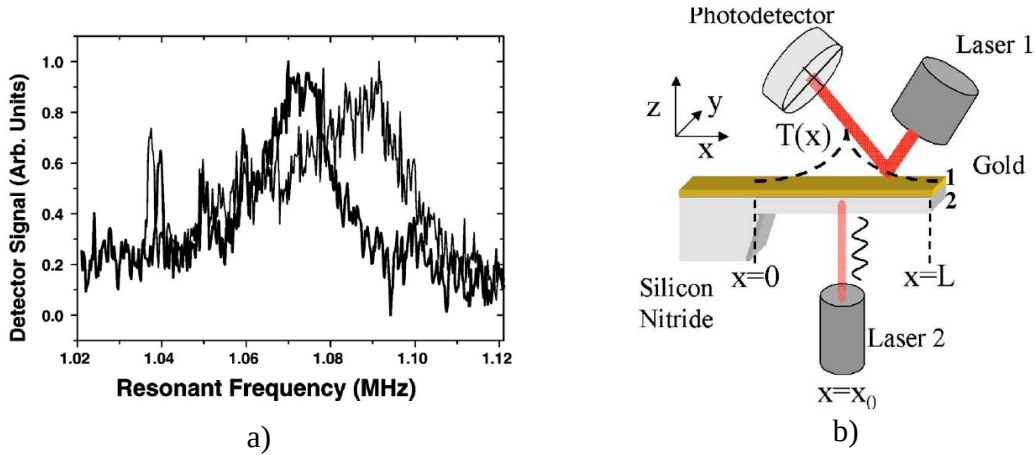


Figure 1.9. The resonance peaks obtained with thermal actuation. a) E-coli detection [30] b) Photo-thermal excitation [68].

Photo-thermal actuation uses a light source (generally laser) to apply heat on the cantilever resulting in a motion at desired frequency. In many devices the cantilever is designed to be biomaterial causing a thermal mismatch as shown in Figure 1.9b [68]. However this is not a requirement to achieve photothermal actuation [69]. The main disadvantage of this method is to cause local heating which creates instability during liquid property measurements

Piezoelectric: This method uses a piezo actuator which excites the housing on which the cantilever sensor is mounted (base excitation) or a piezo layer embedded inside the cantilever. The first one is applicable with commercially available silicon microcantilevers and it is easy to implement into a setup and get results in a short time. However their main disadvantages in liquid environments, lots of standing waves are generated through the acoustic stimulation of the medium which creates an additional noise source. Tamayo et. al [70] performed the mechanical-acoustic excitation by a piezoelectric actuator at the base of the commercially available fluid cell. They achieved to detect specific antigens. Martin Hegner's group in Naughton Institute [40] also used piezoelectric base excitation to measure protein-ligand interactions. Their measurement setup is shown in Figure 1.10a. Ghatkesar et. al. from ETH Zurich[71], Boisen Group at TU Denmark [64] and Mutharasan Group at Drexel University[45, 72] also used piezoelectric base excitation for viscosity detection of liquids..

The acoustic stimulation with the base excitation can be avoided by directly driving of the cantilever using a piezoelectric layer. Basically, a mechanical stress generates an electrical potential across a piezoelectric material and vice versa. For high resolution detection of the deflection it is necessary to operate the cantilever in the dynamic mode since the voltage produced by a static force cannot be maintained by the thin film piezoelectric material[73]. Quate Group at Stanford University is one of the first appliers of this kind of operation for Atomic Force Microscopy (AFM) [74]. Bashir Group at Purdue University used piezoelectric actuation and monitored the growth of cells [75]. The Scanning Electron Microscope (SEM) picture of their cantilever is shown in Figure 1.10b. In addition to the cantilevers piezoelectric actuation is being used for various MEMS based bio-chemical sensor [76]. The main disadvantage of the piezoelectric actuation is the electrical isolation requirement of the cantilever when working in liquid media. This results in additional microfabrication complexity and tedious electrical connection requirements.

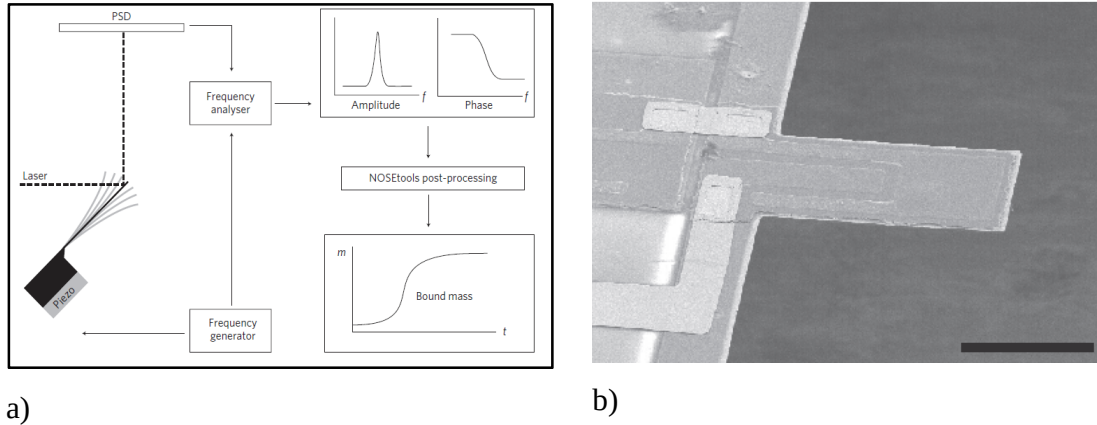


Figure 1.10. a) Piezoelectric base excitation. b) Piezoelectric actuation using embedded piezo layer.

Electrostatic: Although the electrostatic actuation is a common method for MEMS based systems, it is not very preferable for cantilever based biosensors. This is due to the occurrence of bio-chemical sensing in liquid medium. Simply, the cantilevers are made of a conductive material and an AC driven electrode pulls the cantilever. However suspended microchannel resonators (SMR) utilizes this technique [38, 59, 77, 78]. Other than that the electrostatic actuation is not being used in the literature especially for liquid sensing applications.

Electromagnetic: There are mainly two different types of electromagnetic actuation. Lorentz forces can be used for the actuation of a cantilever. A magnetic layer can be evaporated on the cantilever and an external AC magnetic field can be applied [70, 79-84]. Or a conductive pathway on the cantilever can be driven with an AC current under a constant DC external field [46, 50, 51, 53, 85-87]. This type of actuation is also common for viscosity and density monitoring applications. Tamayo et. al. used an electro-coil to actuate a permalloy coated cantilever to detect specific antigens as shown in Figure 1.11a [70]. Lin group at Columbia University also embedded a permalloy layer into the

cantilevers structure and applied an AC magnetic field for actuation [83, 84] (Figure 1.11b). Dufour group at Bordeaux University [46, 50, 51, 53] and Dual group at ETH Zurich [85, 86] uses a conductive pathway inside the cantilever (Figure 1.11d). Both of them use their system for viscosity measurements. It should be noted that the dimensions of the cantilevers in their systems are in mm range.

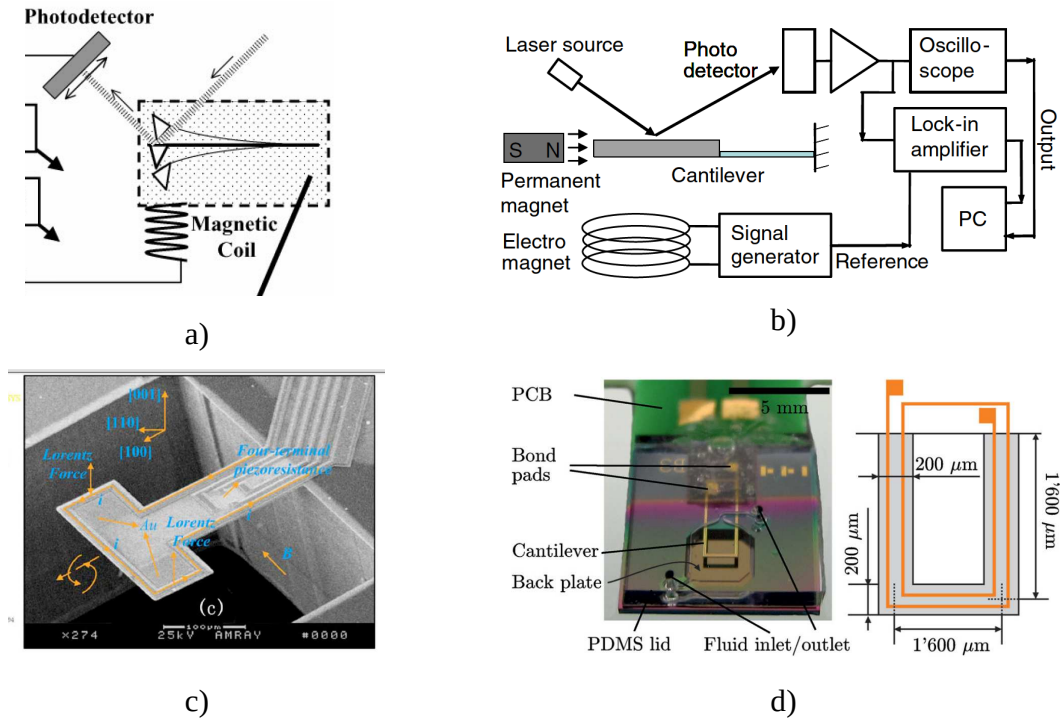


Figure 1.11. Magnetic actuation a) Magnetic surface coated cantilever[70] or b) embedded permalloy layer for excitation with external AC magnetic field[83]. c) Torsional cantilever with embedded conductive pathways [88]. d) Lorentz force based bending mode cantilever [85].

1.2.2.2 Read-out Methods

In order to measure the real-time deflection of cantilevers different sensitive displacement sensors have been used in the literature. Some mature methods are robust and

well established but rather bulky, whereas other techniques are a bit more immature but with the promise of becoming miniaturized [73].

Optical: Optical lever is one of the most commonly used optical read-out system. In most of the commercial AFMs there is a built-in optical lever setup. The main principle is as follows, a laser beam is focused on the cantilever which acts as a mirror. The laser light is reflected on a position sensitive photodetector and the intensity changes are detected (Figure 1.12a). However usage of a reference cantilever is challenging with this setup. The multiplexing with this method is achieved with a unique design created by IBM Zurich Lab. [89], an array of eight VCSELs (vertical cavity surface emitting lasers) were focused on 8 cantilevers and the reflected light was collected by a single photodetector (Figure 1.12b). It is capable of tracking individual movement of the spots coming from each cantilever.

Another common optical read-out is based on interferometry. This method is also used with some applications of this thesis and the detailed explanation can be found in 2.3.2.1. In this method diffraction gratings integrated with the tip of the cantilevers partially reflects and passes the light. The passed light reflects from the bottom surface and interferes with the reflected light. The motion of the cantilever results in variations in the intensity. The frequency of these variations equals to the frequency of the cantilever vibration. This type of read-out is commonly used in different types of MEMS structures such as capacitive micromachined ultrasonic transducers (CMUTs) [90] and thermo mechanical detectors [91], AFM cantilevers to achieve high-bandwidth force measurements as shown in Figure 1.12c [92]. Also in the previous studies of our group about hepatitis virus detection this method is utilized [43, 44].

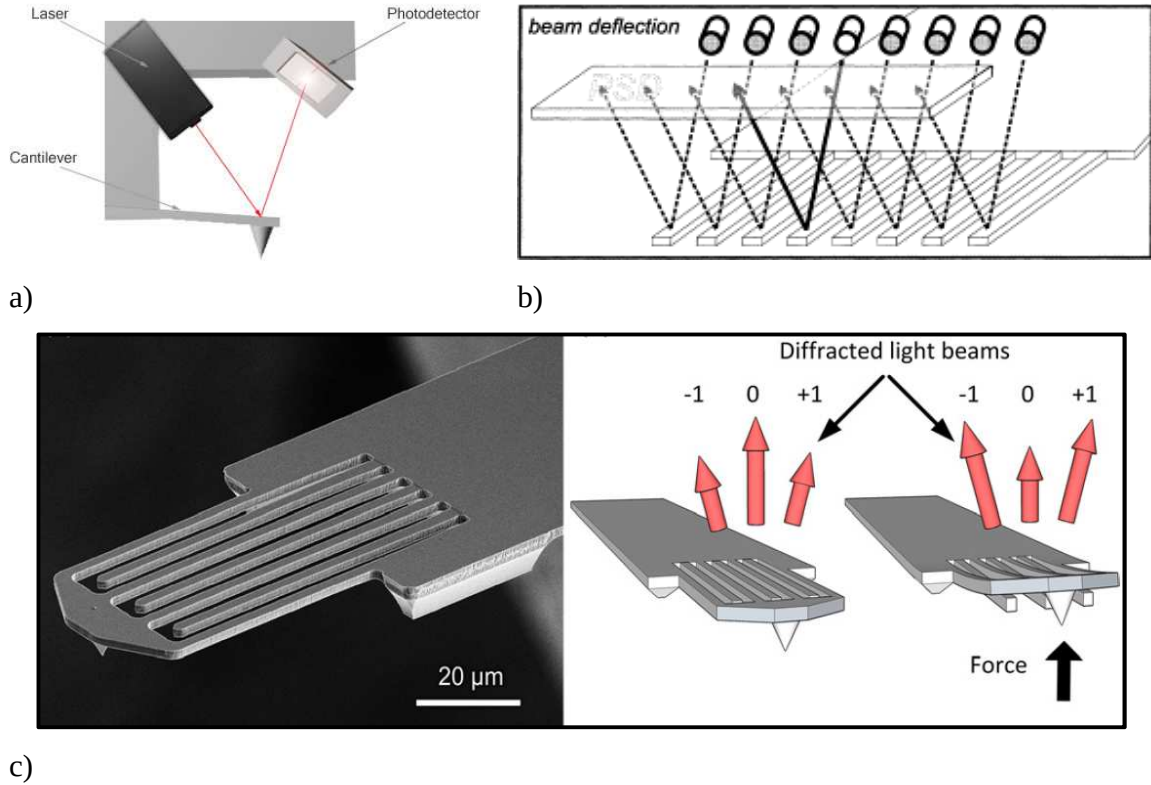


Figure 1.12. a) Optical Lever Method. b) IBM cantilevers using a VCSEL array for multiplexing [89]. c) AFM tip with integrated interferometric read out[92].

Piezo: Piezoelectric and piezoresistive materials have been used as a common read-out technique in addition to their usage for actuation. The piezoelectric read-out have been used since 1993[93]. Afterwards many groups have been implementing the technique for cantilever-based sensing. Mainly the cantilevers are coated with lead zirconate titanate (PZT) or zinc oxide. Mutharasan group at Drexel university detected waterborne parasite *Giardia lamblia* using millimeter sized glass cantilevers coated with PZT [39]. PZT coated Silicon nitride cantilevers have been introduced by the group of Tae Song Kim at KAIST and used for many biosensing applications including Hepatitis B virus DNA [94] at femtomolar concentrations. Seon Hwang's group again from KAIST used PZT read-out for

viscosity and density detection of blood serum [95]. Aluminum nitride is also a piezoelectric material having high stiffness and capability of epitaxial growing on single-crystal silicon. Main usage area of this material is surface acoustic wave devices [73]. The main challenge of the piezoelectric techniques is challenging fabrication of these materials. In addition they are not generally clean-room compatible. It is easy to scale this technique to multiple arrays.

Piezoresistive read-out cantilevers have an integrated resistor with piezoresistive properties. With the piezoresistive property the resistance of the material changes with the cantilever bending. Thus, by measuring the resistance change the deflection of the cantilever can be determined. Figure 1.13 shows a schematic representation of the measurement principle. Several groups work on the development on piezoresistive read-out [96, 97]. Also the method is utilized with nanometer scale cantilevers to achieve sensitive mass detection up to 1 ag by Roukes group at California Institute of Technology [98]. The method is able to work in both liquid and gas phase and it is highly suitable for multiplexing. Like in piezoelectric actuation, it is necessary to isolate the conductive wires and layers for operation in conductive liquids.

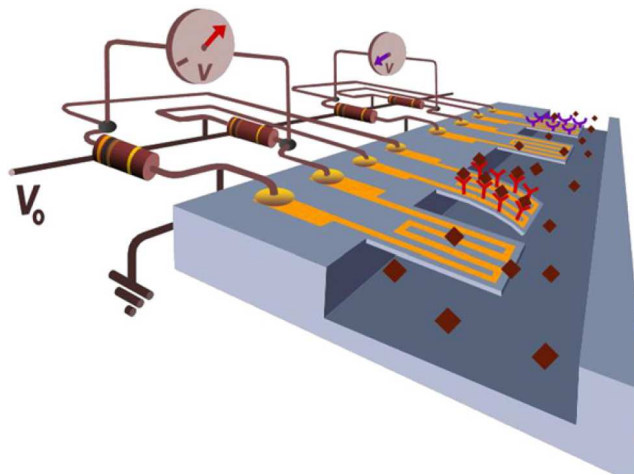


Figure 1.13. Schematic drawing of the piezoresistive read-out. When molecules bind to the cantilever, bending occurred is detected as a change in the resistance of the resistor embedded inside the cantilever. The method allows multiplexing and reference cantilever usage [73].

Capacitive: When a cantilever is placed between two parallel electrodes the cantilever deflection will cause a capacitance change. This integrated read-out was first introduced for AFM probes [99] and has later also been implemented in cantilever-based sensing [100]. Today, capacitive read-out is mainly explored for mass detection in non-liquids.

Pick-up coil: Pick-up coils are rarely used with cantilever-based sensors compared to other read-out methods. The cantilever should be made of magnetic materials. Puckett et al. utilized pick-up coils with magnetoelastic cantilevers and measured coagulation times [101, 102]. However the pick-up coils are bulky and prone to environmental noise.

1.2.3 State of the Art in Cantilever Based Sensing Technologies

We presented the recent literature about mechanical biosensors dealing with mass detection and viscosity density sensing applications. Although the recent achievement of the technology for mass and liquid property sensing is shown in details in the previous sections it will be better to summarize and to clarify the state of the art.

For **viscosity and density sensing** with micro/nano mechanical sensors the most important metrics are the sensitivity and accuracy. The accuracy is mainly reported with the percent error between the measurements with the proposed system and the reference laboratory devices [103, 104]. As we will discuss in details in section 3.1, these devices are bulky and are not suitable for point-of-care use and biological applications since they require high sample volumes and does not have disposable cartridges. With the cantilever-based systems Rust et al. [85] achieved the measurement accuracy of 0.4% for density in 990-1050 kg/m³ range and 6% for viscosity for 0.94-2 mPa s range. Bircher et al. [69]

reported the viscosity with 10 % accuracy and density with 4% accuracy for the ranges 1-10 mPa s and 998 to 1176 kg/m³, respectively. They published their works in 2013. Also in another very recent study, Manzanque et. al. used a piezoelectric MEMS resonator [105] and achieved the measurement accuracy of 0.4% for density in 680-905 kg/m³ range and 8% for viscosity for 0.4-7.4 mPa.s range. They report a minimum detectable viscosity of 2.07×10^{-3} mPa.s and a density of 4×10^{-3} kg/m³. Also there is no reported study on measuring blood plasma with cantilevers except ours.

For the **coagulation time measurements** which are well-know and common Point-of-Care Tests, the most important parameter is the accuracy and the reliability of the sensors. The time resolution or sensitivity is not important parameters. The device should avoid false positives or false negatives and can clearly distinguish the normal and abnormal situations. There are commercial devices already in use [18, 106] satisfying most of the needs of the market. There are MEMS and microfluidics based sensors reported in the literature which are analyzed in details in section 5.1. Shih et al. [107] conducted clinical tests with a microfluidic device and reported 5% accuracy with the reference device for PT tests. However no group ever used microcantilevers for coagulation time testing. Another parameter is the sample volume required. In most of the literature 10 μ l volume requirement is satisfied for testing [16].

About **biological mass detection** with cantilever based sensors Tamayo et. al.[5] published a review paper in 2013 and collected the minimum detected masses in vacuum, liquid and air:

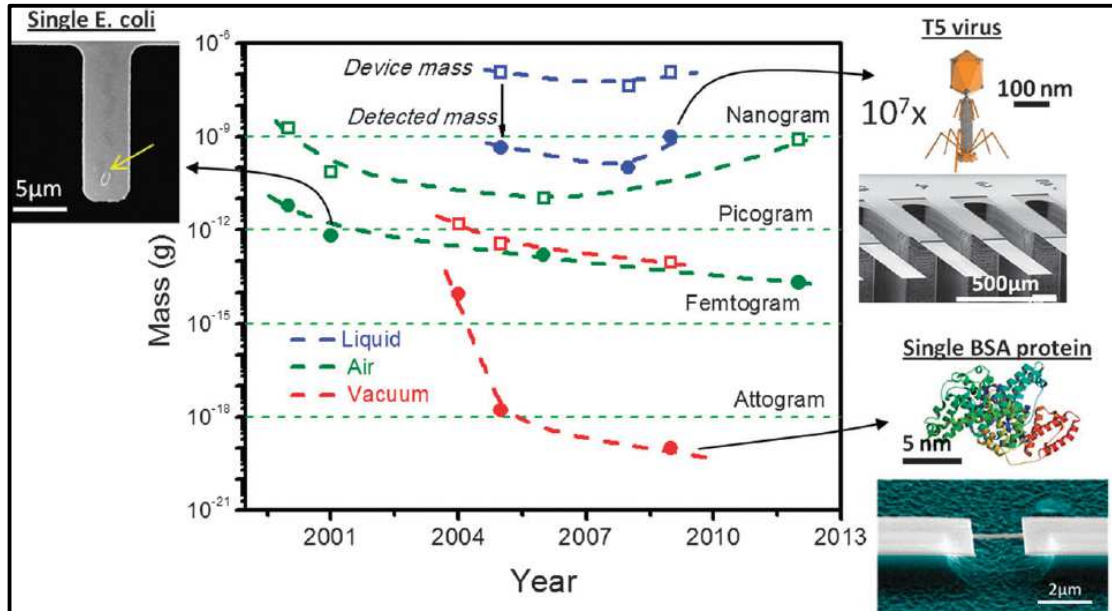


Figure 1.14 . The chronological achievements in label-free biological detection base on nanomechanical resonators. The device mass and the detected mass levels are plotted as empty and filled symbols, respectively [5].

According to the chart for in liquid operation the Hegner group at University of Basel achieved to measure up to 1.5 pM in-liquid for virus detection (T5 virus), (Figure 1.6b). The minimum mass detected is 4 ng in their studies [40-42]. With the vacuum and air media very small masses are achieved [108] up to single molecule, but this thesis doesn't deal with vacuum or air operation cases.

1.3 Contributions of this research

Main contributions of this thesis research to the literature and the publications resulting from the thesis are summarized. Other relevant studies I contributed during my PhD studies are also mentioned.

Microcantilever-based sensor developed in this thesis is utilized for blood plasma viscosity measurements as presented in detail in Chapter 3. Major tasks involved scale up of the process from chip scale to wafer scale for repeatable results, integration of cantilevers within fluidic cartridge, and the development of detailed cartridge preparation and data acquisition protocols. The results are presented in comparison with the reference laboratory equipment. Two conference papers (C5 and C7) and one journal paper which is *featured as “Cover Article”* are published out of this study (J4). One of the conference papers is also published in a journal (J5). These were the first studies utilizing cantilevers for blood plasma viscosity measurements.

As reported in Chapter 4, a novel practical method is developed to measure viscosity and density of fluids using cantilevers. The method utilizes multiple cantilevers with different widths operated in the same fluidic channel. This enables simultaneous and real-time viscosity and density measurements with high accuracy and resolution beyond the state-of-the-art. A manuscript is submitted to a journal within that study (J1).

In Chapter 5, the design of the sensor is modified after various fabrication and design iterations in order to obtain multiplexed plasma coagulation time measurements. It is very challenging to attain a repeatable protocol for this type of measurements. For this purpose new protocols for cartridge functionalization and measurement are developed. This is the first study utilizing dynamic cantilevers for coagulation measurements. Three conference papers (C1, C3 and C4) in prestigious conferences and one journal paper (J2) which is *featured as “Hot Article”* is published within this study.

In Chapter 6, the investigation of the system for biological mass detection is reported. The setup has multiple channels in parallel and the results of different antigen detection experiments are presented. A TUBITAK 1001 project, 111E184, about antigen detection is successfully completed. Our group published several papers on antigen detection prior to this thesis research and recent results were geared more towards repeating the

measurements in the newly developed cartridge rather than the more controlled environment used previously.

An additional study, not reported in this thesis, was conducted to use the cantilevers for gas sensing applications. I mainly contributed to the fabrication and measurements. A journal paper (J3) and a conference paper (C6) were published within that work. In addition I conducted fabrication of MEMS-based scanners with piezoelectric actuation, which resulted in a conference paper (C2).

Overall 4 journal papers have been published, and 1 other is under review. In addition a review paper on cantilever based sensors for liquid property monitoring applications is being prepared. We had a chance to present our work in 7 refereed international conferences. You can find a bibliography entitled as “Publications Resulting from This Thesis Research” listing all the publications out of my PhD studies.

2 MICROFABRICATION AND MEASUREMENT PRINCIPLE

This chapter provides the details of the theory and introduces all the main components of the developed dynamic cantilever sensor system. Section 2.1 provides theory of operation in liquid medium. Section 2.2 provides microfabrication details and the process improvements such as the scale up to wafer scale operation, section 2.3.1 and section 2.3.2 provides details of the magnetic actuation and the optical sensor, respectively, and section 2.3.3 provides the details of the post-detection electronic readout using resonant frequency tracking and phase tracking methods.

2.1 Theory of Cantilever Dynamics

In the theory part the mechanical behavior and vibration of cantilevers will be studied in under damped media (such as in air) and almost over damped media (such as in liquids). The establishment of the well-known formulations will be shown. First the vibration characteristics of a cantilever beam will be shown with fundamentals of mass detection principle. Afterwards the analytical expressions of dynamics of the cantilevers inside viscous media will be shown.

2.1.1 Resonant behaviour of cantilever beams

The main parameters defining the resonant characteristics of a mechanical structure are the spring constant, resonance frequency, and corresponding quality factor. One-dimensional (1D) Euler–Bernoulli assumption for an initially flat, thin, homogeneous cantilever beam can be used to express the time-varying displacement w across the cantilever [109] :

$$\hat{E}I \frac{\partial^4 w(x, t)}{\partial x^4} + (\rho A + \chi) \frac{\partial^2 w(x, t)}{\partial t^2} + \xi \frac{\partial w(x, t)}{\partial t} = q(x, t) \quad (1)$$

$\hat{E}$ is the apparent Young's modulus and can be expressed as $E/(1 - \nu^2)$ with E being Young's modulus and ν being the Poisson ratio. ρ is the specific mass density of the beam material, hw is the cross sectional area (A) (illustrated in Figure 2.1), I is the moment of inertia $wh^3/12$, ξ is the damping coefficient per unit length per unit velocity, χ is the additional mass per unit length, and $q(x, t)$ represents the forcing function in N/m . The added mass χ can either be the adsorbing molecules on the surface or it may be the mass of the surrounding media molecules that are accelerated by the vibration of the beam. This leads to a dependency of the resonance frequency on the resonance behavior in general from the surrounding media. The Euler-Bernoulli approach doesn't include the effects of rotary inertia and shear deformations and assumes small vibration amplitude (i.e., neglects the non-linear terms due to large vibration amplitude) [109].

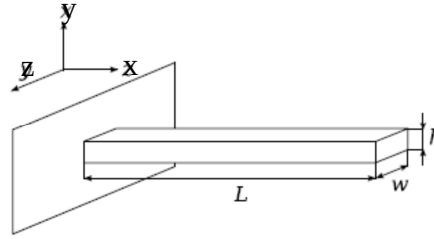


Figure 2.1. Cartoon drawing of a cantilever beam

Equation (1) is a differential equation with two variables: the time variable is mainly related with the frequency and damping characteristics and the position variable gives the displacement characteristics across the beam and defines the shape of the vibration mode. The mode shape functions correspond to the relative movement of different points of the beam. These variables can be separated using a function:

$$w(x, t) = W(x)T(t) \quad (2)$$

For the case without additional driving load ($q(x,t)=0$) this leads to the following equation for the Eigen frequency ω_0 [110]:

$$\frac{\hat{E}I}{(\rho A + \chi)} \frac{\frac{\partial^4 W(x)}{\partial x^4}}{W(x)} = -\frac{\xi}{(\rho A + \chi)} \frac{\frac{\partial T(t)}{\partial t}}{T(t)} - \frac{\frac{\partial^2 T(t)}{\partial t^2}}{T(t)} \equiv \omega_0^2 = \text{const.} \quad (3)$$

The time variable can be written as:

$$\frac{d^2 T(t)}{dt^2} + \frac{\xi}{(\rho A + \chi)} \frac{dT(t)}{dt} + \omega_0^2 T(t) \equiv \text{const.} \quad (4)$$

The time dependence can be expressed like a damped 1D harmonic oscillator:

$$T(t) = B e^{-\gamma t} \sin(\omega_0 t + \varphi) \quad (5)$$

The position variable can be written as:

$$\frac{\hat{E}I}{(\rho A + \chi)} \frac{\partial^4 W(x)}{\partial x^4} - \omega_0^2 W(x) = 0 \quad (6)$$

The solution of a homogenous fourth-order differential equation is the sum of four linearly independent solutions:

$$W(x) = c_1 \cos\left(\frac{\lambda}{L} x\right) + c_2 \sin\left(\frac{\lambda}{L} x\right) + c_3 \sinh\left(\frac{\lambda}{L} x\right) + c_4 \cosh\left(\frac{\lambda}{L} x\right) \quad (7)$$

The boundary conditions of the cantilever beam should be applied to find the coefficients c_1, c_2, c_3, c_4 :

$$\begin{aligned} W(0) = 0, \quad \left. \frac{dW(x)}{dx} \right|_{x=0} = 0, \quad \left. \frac{d^2 W(x)}{dx^2} \right|_{x=L} = 0, \\ \left. \frac{d^3 W(x)}{dx^3} \right|_{x=L} = 0, \end{aligned} \quad (8)$$

L is the length of the cantilever, and the non-dimensional λ is defined by:

$$\left(\frac{\lambda}{L}\right)^4 = \frac{\rho A \omega_0^2}{\hat{E} I} \quad (9)$$

The first two boundary conditions are related with the clamped end of the cantilever. The third one is due to the lack of bending moment at the free end of the cantilever, and the fourth excludes any torsion at the tip. With these boundary conditions, a characteristic equation can be calculated for cantilever beam:

$$\cos \lambda \cosh \lambda = -1 \quad (10)$$

The discrete values of λ_a in Equation (11) are depending on the mode number. The λ values for the first four modes are [109]: $\lambda_1=1.875$, $\lambda_2=4.69$, $\lambda_3=7.86$, $\lambda_4=11.00$. When these parameters are inserted into Equation (9), the resonance frequency of the cantilever beam for the undamped case can be calculated as:

$$\omega_{a,0} = 2\pi f_{a,0} = \frac{\lambda_a^2 h}{\sqrt{12} L^2} \sqrt{\frac{E}{\rho(1-\nu^2) + \chi}} = \frac{\lambda_a^2}{L^2} \sqrt{\frac{\hat{E} I}{\rho A + \chi}} \quad (12)$$

And the quality factor is:

$$Q = \frac{\omega(\rho A + \chi)}{\xi} \quad (13)$$

The resonance frequency of a cantilever for the damped case can be expressed as:

$$\omega_a = \omega_{a,0} \cdot \left[1 + \frac{1}{4Q^2}\right]^{-1/2} \quad (14)$$

For $\xi=0$ there is no damping and the amplitude at resonance goes to infinity. Also from (13) it can be seen that Q-factor is proportional to the resonance frequency. The Q and resonance frequency will be revisited and modified for the liquid operation case in section 2.1.2. The system can also be modeled as a spring mass system as shown in Figure 2.2.

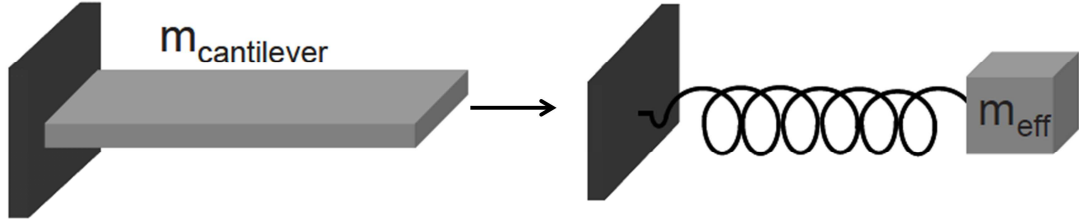


Figure 2.2. Cantilever as a spring-mass system (1D harmonic oscillator)[111]

The resonance frequency of this system is:

$$f = \frac{1}{2\pi} \sqrt{\frac{k}{m \cdot n}} = \frac{1}{2\pi} \sqrt{\frac{k}{m_{eff}}} \quad (15)$$

n is a geometrical parameter. For the fundamental mode of a rectangular cantilever beam $n=0.2427$. m_{eff} is the effective mass, and k as the force constant or stiffness:

$$k = \hat{E} \left(\frac{h}{L} \right)^3 \frac{b}{4} \quad (16)$$

A mass uniformly bonded over the surface can be correlated with a frequency change by:

$$\Delta m = -\frac{k}{4\pi^2 n} \left(\frac{1}{f_0^2} - \frac{1}{f_m^2} \right) \quad (17)$$

Where f_m is the loaded beam frequency:

$$f_m = \frac{1}{2\pi} \sqrt{\frac{k}{m_{eff} + n\Delta m}} = \frac{f_0}{\sqrt{1 + \frac{\Delta m}{m}}} \quad (18)$$

By applying a first order Taylor series expansion:

$$f_m \approx f_0 \left(1 - \frac{1}{2} \frac{\Delta m}{m}\right) \Rightarrow \frac{\Delta f}{f_0} \approx -\frac{1}{2} \frac{\Delta m}{m} \quad (19)$$

The minimum detectable mass can be calculated from the minimum detectable frequency change. Different parameters affect the frequency stability of a cantilever. The most dominant is the noise acting on the resonator. The second one is the ability of the structure to store the energy which is related with the damping. The minimum detectable frequency shift $\delta\omega$ of a cantilever under the influence of thermal noise $k_B T$ can be estimated as [112]:

$$\delta\omega = \sqrt{\frac{\omega_0 k_B T B}{k Q \langle A^2 \rangle}} \quad (20)$$

where B , k , k_B , and T denote the bandwidth, spring constant, Boltzmann constant and absolute temperature, respectively. $\langle A^2 \rangle$ is the mean-square vibration amplitude. It is obvious that the quality factor, which is related to damping, is a key parameter.

Until now the general expressions about the cantilever resonance behavior was presented. Since the cantilevers used in this thesis are operated inside viscous liquid medium, certain hydrodynamic forces act on the cantilever. Hence the expressions should be modified in the light of the theoretical assumptions emerged in the last decades.

2.1.2 Cantilevers operated in liquids

The cantilever beam structure is considered as Euler-Bernoulli beam. When the cantilever is operating in viscous media, a hydrodynamic force per unit length, F_{fluid} , is applied to the structure [36]. This force includes two different terms [53]:

$$F_{hydro} = -g_1 \dot{y} - g_2 \ddot{y} \quad (21)$$

$y=y(x, t)$ is the deflection function of the beam axis. The first term is the viscous drag force and the second term is the inertial force related to the liquid mass accelerated by the

cantilever as shown in Figure 2.3a. According to Sader's theory, [36] the liquid vibrating with the cantilever can be assumed as a cylinder with mass per length as given by g_2 . g_1 and g_2 can be expressed as [54]:

$$g_1 = M_L(2\pi f_L) \left(b_1 \frac{\delta}{w} + b_2 \left(\frac{\delta}{w} \right)^2 \right) \quad (22)$$

$$g_2 = M_L \left(a_1 + a_2 \frac{\delta}{w} \right) \quad (23)$$

where w is the width of the cantilever, f_L is the damped resonant frequency of the cantilever in liquid. $a_1=1.0553$, $a_2=3.7997$, $b_1=3.8018$, and $b_2=2.7364$ are dimensionless "Maali's parameters" for an infinitely thin beam [113]. $M_L = \pi \rho_f w^2 / 4$, where ρ_f is the fluid density. The illustration in Figure 2.3b shows the δ parameter, which is the fluid layer thickness where the fluid velocity drops by a factor of $1/e$. δ , can be expressed using the viscosity of the fluid (μ), as:

$$\delta = \sqrt{\frac{2\mu}{\rho_f(2\pi f_L)}} \quad (24)$$

One can see that this term includes inertial and viscous forces; actually this is another form of the Reynolds number normalized with the width of the structure:

$$Re = \frac{\rho_f(2\pi f_L)w^2}{4\mu} \quad (25)$$

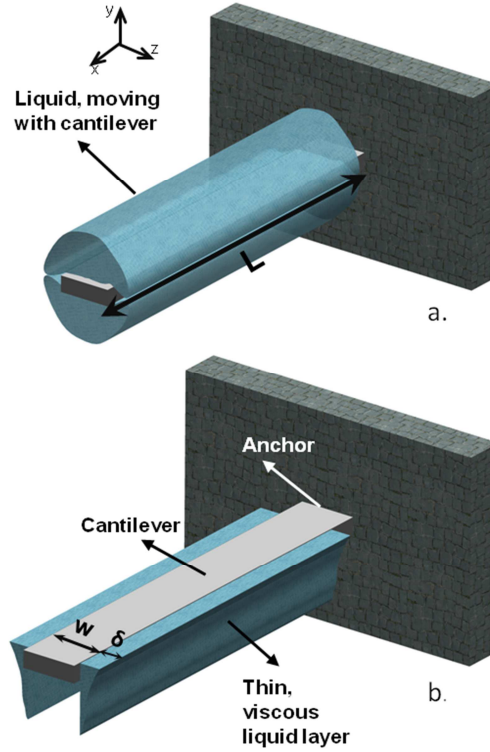


Figure 2.3. a) The illustration of the liquid mass, Lg_2 , accelerated by the vibrating cantilever. b) Thin viscous layer, δ , where fluid velocity dropped by a factor of $1/e$ during cantilever motion.

The Reynolds number of the system should be smaller than 1000 and larger than 0.1 to use these expressions. For the cantilevers used in this thesis the Reynolds numbers vary between 1 and 300 depending on the cantilever width ($5\mu\text{m}$ to $100\mu\text{m}$) and the liquid viscosity (1 mPa.s-20 mPa.s).

The resonant frequency and Q including the hydrodynamic force effects in liquid can be expressed as:

$$f_L = f_0 \frac{1}{\sqrt{1 + Lg_2/m}} \sqrt{1 - \frac{1}{2Q^2}} \quad (26)$$

$$Q = 2\pi f_0 \frac{\sqrt{1 + Lg_2/m}}{Lg_1/m} \quad (27)$$

L and m are the length and mass of the cantilever respectively, and f_0 is the undamped resonant frequency in vacuum, which is very close to that of air.

Frequency shift due to viscosity and density changes can be computed iteratively using the above equations. The known values for fluid density and viscosity, and resonant frequency in air, f_0 , are substituted in Eqs. (22), (23), (24) and (26) to calculate δ , g_1 and g_2 , and f_L . As can be seen from Equations (24) and (26); δ and f_L are dependent on each other. Hence an iterative solution is required to obtain these values. The resonant frequency in air, f_0 , is chosen as the initial value of f_L . The iteration continues until the difference is less than 1e-4 Hz between two successive values of f_L . The number of iterations required to converge is about 9. During the study these analytical results are used for prediction of the response of cantilevers to viscosity and density changes. The results obtained with these assumptions are presented in Figure 3.3.

Both the f_L and Q are coupled functions of viscosity and density. Hence it is challenging to discriminate the effect of them. A novel method is developed for this purpose as will be detailed in chapter 4.

The variations of the liquid viscosity and density will affect both the resonant frequency and Q . This also results in a change in the phase delay between input and output signals. Hence the frequency value corresponding to a constant phase delay will change or vice versa. These changes can be tracked with electronic read-out methods as described in section 2.3.3. The amount of the change can be related with the viscosity values after some calibration steps.

2.2 Microfabrication

First we introduce the details of the previous work by researchers at Koç University Clean Room [114, 115] and then continue with the improvements to the process performed during this thesis research. The developed process is used with slight modifications and editions to produce the cantilevers needed for different parts of this research.

Figure 2.4 shows the microfabrication flow of the cantilevers. In some of the steps the recipes shown in the MS theses of Kilinc [114] and Yavuz [115] are used. The main improvement was carrying up the system to wafer scale fabrication. These improvements are discussed after describing the standard microfabrication steps. First the fabrication scheme will be shown and described briefly. Afterwards the improvements will be treated.

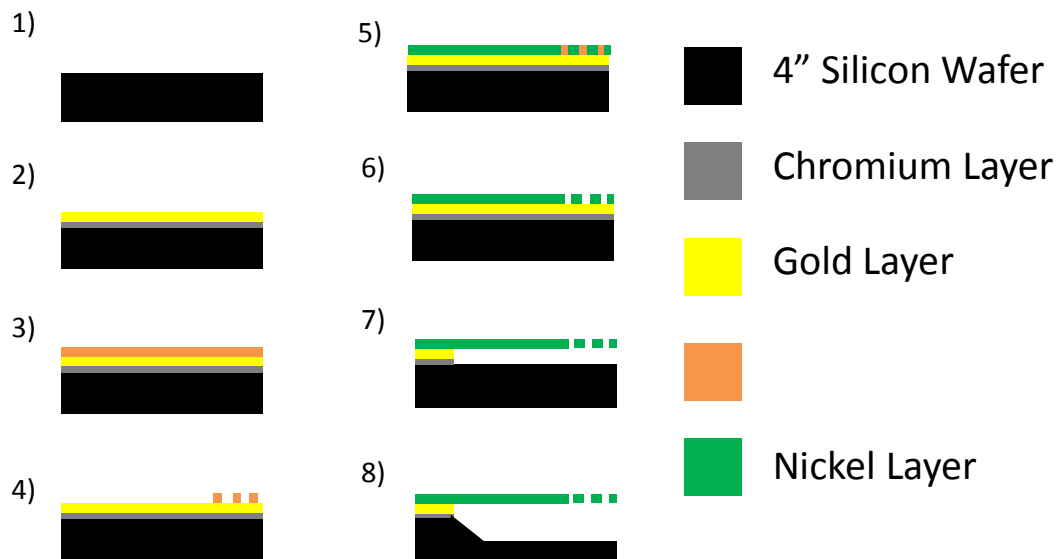


Figure 2.4: The fabrication flow

A 4" diameter, <100> single crystalline silicon prime wafer (with thickness and resistivity values of $400 \pm 25 \mu\text{m}$ and $0.1\text{-}0.5 \Omega \text{ cm}$, respectively) is used. Cleanness of silicon wafers prior to fabrication is ensured with the standard (RCA1) cleaning at 80°C for 10 minutes followed by the diluted HF (5%) treatment to get rid of organic contamination and oxide layer on the surface, respectively. **(Step 1)**. A 20 nm/100 nm Cr/Au layer is deposited on a 4" diameter, <100> single crystalline silicon prime wafer) using RF sputtering with Vaksis PVD Handy sputter tool **(Step 2)**. Sputtering is conducted inside Ar plasma at $1.5\text{e-}5$ Torr vacuum. The Cr target (K.J Lesker Inc. part no: EJTCRXX353A2) used during sputter has a 3" diameter, 0.125" thickness and 99.95% purity whereas the Au target (K.J Lesker Inc. part no: EJTAUXX403A2) has same dimensions and 99.99% purity. Here Cr layer is used to provide adhesion of gold layer to Si, whereas Au layer serves as the seed layer for subsequent Ni electroplating. A positive photoresist (PR), AZ1514H[®] (*Microchemicals GmbH*) with a thickness of 1500nm, layer is coated on the top of Au surface with SPS Spin150 manual spin-coater. **(Step3)** Immediately after spin-coating, the photoresist-coated wafer is soft-baked on a hot plate at a temperature of 100°C for 2 minutes. During soft-bake process, the wafer is kept covered for preventing the heat loss and effect of light on the photoresist. Soft-bake is needed for the proper sticking of photoresist on the wafer. After baking, the cantilever geometries are patterned with UV lithography using Karl-Süss MA45 mask aligner. Inside the mask aligner, the wafer is exposed to UV light whose intensity is 22 mW/cm^2 . The exposure time is determined as 17 seconds with contact printing option. Afterwards the development with AZ351B Developer (*Microchem.*) is conducted which takes 1 minute. **(Step 4)**. In the previous theses using a similar fabrication flow [114, 115], the wafers were scribed and diced at this level and the electroplating conducted at the chip scale. This was a very time and labor consuming process. Also due to the electrode conditions the thickness variations on the chip used to occur. Hence at this step a custom

made 4" wafer scale electroplating setup is built up. The details of this setup will be shown in 2.2.1. Electroplating of nickel is conducted with a custom made setup using a nickel sulphamate bath at 45°C with 5 mA/cm² current density. **(Step 5)** The bath is composed of 600 g/L nickel sulphamate Ni(SO₃NH₂)₂·4H₂O) (Sigma Aldrich 262277), 10 g/L nickel chloride (NiCl₂·6H₂O) (Alfa Aesar A14366) and 40 g/L boric acid (H₃BO₃) (Alfa Aesar A16624). An Inco 4"x4" type Nickel anode with a purity larger than 99.9% is used during electroplating.

After the electrodeposition of nickel layer, the remaining PR is stripped via AZ100 Remover (Microchem.) **(Step 6)**. Wet etching of Au and Cr are conducted with 1:3 dilution of Transene Inc. GE- 8148 type gold etchant and 1:3 dilution of Microchrome Inc. CEP-200 chromium etchant at room temperature in 90s and 75s, respectively. **(Step 7)** Finally, cantilevers are released through wet etching of Si in 35% KOH solution (Sigma Aldrich) at 60°C in 45 minutes (Step 8). Anisotropic wet etching of silicon is carried out to a depth of 15 µm to avoid squeeze film damping effect. At the end of the fabrication flow, the wafer or chip is immersed in water, acetone and IPA subsequently for at least 5 minutes and dried at room temperature on a flat surface to prevent stiction of microcantilevers as much as possible.

Different types of cantilevers with different geometries are fabricated with the described process depending on the application. Figure 2.5 shows the close-up micrograph and the 3D drawing of the cantilevers with special diffraction gratings. These cantilevers have a length of 60 µm, excluding the head structure consisting of gratings, a width of 5 µm and a thickness of 1 µm. The diffraction gratings at the tip have 3 µm width, 20 µm length and the grating period is 6µm. Also larger, thicker and wider cantilevers without diffraction gratings are fabricated for specific applications. They will be discussed in the related chapters.

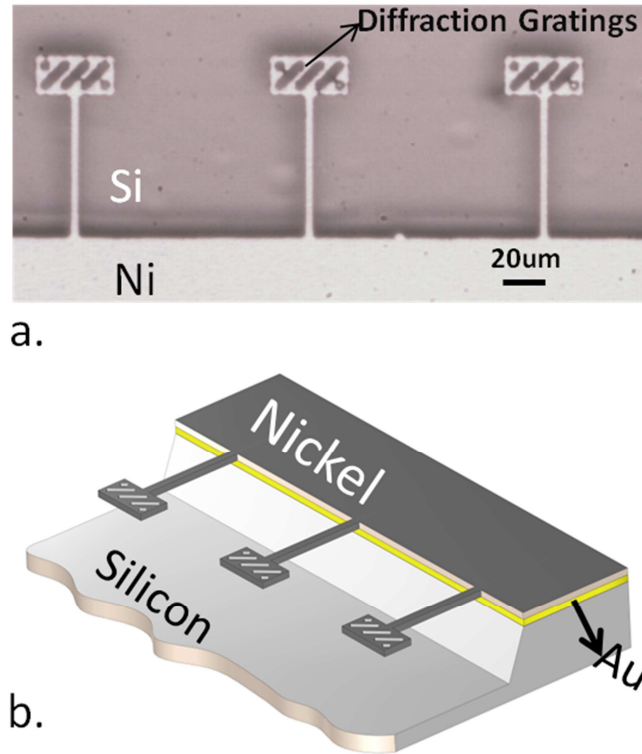


Figure 2.5: a) Micrograph of microfabricated cantilevers with diffraction gratings. b) 3D illustration of the cantilevers, which are 15 μm above the Si surface

After microfabrication, the chip containing the micro cantilevers is attached inside a microfluidic channel. The channels are formed by machining Poly Methyl Methacrylate (PMMA) with CNC. Different types of microfluidic channels using biocompatible tapes are also used for different applications. Although the main principle remains similar, different channel geometries and packaging components are utilized for specific applications. These variations based on the applications will be treated in detail in the upcoming chapters, specifically in sections 4.2.1, 5.2.1, 6.2.1. Figure 2.6 shows one of these cartridge designs.

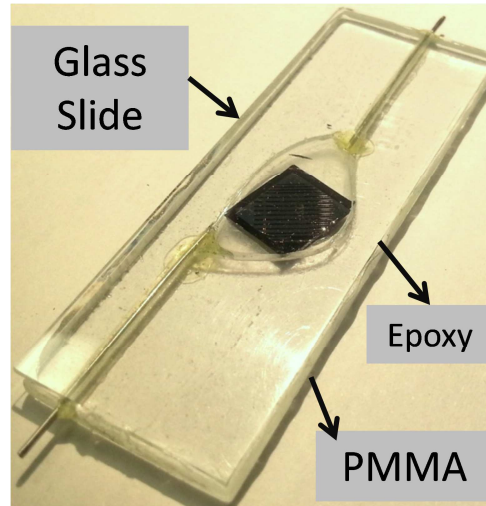


Figure 2.6: The PMMA cartridge

2.2.1 Wafer Scale Electroplating Setup

In the previous theses [114, 115] using a similar fabrication flow, the wafers were scribed and diced after photolithography and the electroplating conducted at the chip scale. In order to get valuable biological results, many tests should be conducted to present the reproducibility of the system. This creates a need for an extensive sensor supply especially when a MEMS sensor is not reusable and wasted per single experiment. The chip level electroplating and subsequent etching processes were time consuming and labor intensive. Chip level process was the main bottleneck in getting repeatable and fast results. . Furthermore, due to the electrode conditions the thickness variations on the chip used to occur, which created a non-standard profile and affected the repeatability of the biological results. Hence at this step a custom made 4” wafer scale electroplating setup is built. The photos of the old chip scale and wafer scale setups are shown in Figure 2.7 and Figure 2.8, respectively.

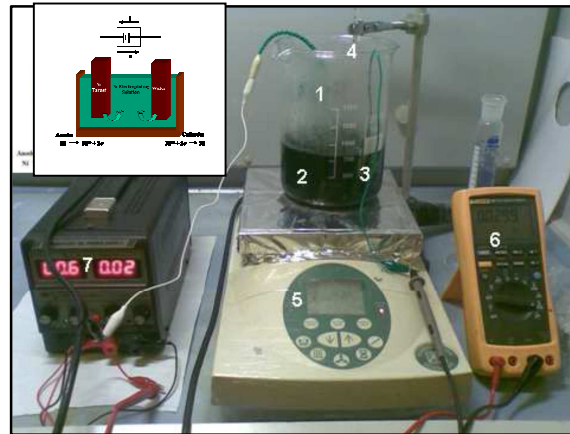


Figure 2.7: Electro-plating process setup: 1) Bio-sensor chip attached with four crocodile cables from four contact places, 2) Nickel Sulphamate solution, 3) Nickel Target Anode, 4) Thermometer for measuring the solution temperature, 5) Magnetic heater for adjusting temperature and stirring solution, 6) Digital Multimeter for monitoring the current, 7) Power Supply for controlling the voltage and current. [115]

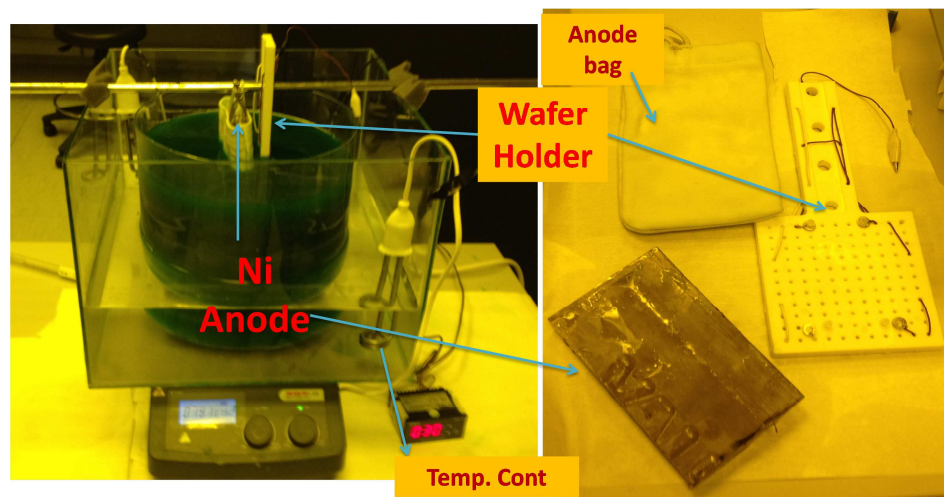


Figure 2.8: The wafer scale electroplating setup. The Teflon wafer holder places the electrical connections; the Ni anode is kept inside the bag to prevent contamination.

In the wafer scale setup the electrical connections were done to the chips with crocodiles which resulted in significant thickness variation at adjacent regions to a contact point [114-116]. In the new electroplating setup the volume of the solution is increased 0.25 lt from 1 lt. A large nickel anode is used with an anode bag to prevent contamination. A water bath outside the electroplating bath is facilitated for heating and temperature control. The stirring of the bath is done with the stirrer underneath the water bath. A custom made Teflon wafer holder is used for holding the wafer and housing the electrical connections. A photo of the electroplating setup and the components is shown in Figure 2.8. Both the anode and the wafer hang on a rod to be kept aligned. Also their position and the gap between them can be adjusted. With this setup the overall process speeded up significantly. With the die level electroplating the thickness variations on one MEMS die was in the order of ± 100 nm [116]. With the wafer level electroplating, thickness variation reduced to ± 150 nm across the full wafer and to ± 10 nm across one chip, which improved the uniformity and repeatability substantially.

2.3 Measurement Setup

2.3.1 Magnetic Actuation

The nickel cantilevers are actuated magnetically with a coil attached underneath the PMMA flow cell. A pair of bar (3 x 4 x 9mm) neodymium permanent magnets are placed parallel to the cantilever in order to generate DC magnetic field (0.5 mT) and saturate the magnetization of the Ni cantilevers. When the cantilevers are magnetized with the DC field, a larger displacement can be achieved using the AC coil [117]. For minimizing parasitic capacitive effects and electric resonances in the coil itself, a low turn coil ($N=80$) is used. The total diameter and height of this custom made coil is 14 mm and 12 mm respectively, whereas the wire diameter is 0.3 mm. (Figure 2.9a) The coil is driven with a

high power broadband current amplifier with a fixed current of $1A_{p-p}$ over the whole frequency range. This circuit is capable of driving from DC to 70 kHz. Since no direct electrical contacts are required on the chip, magnetic actuation with an external coil has a significant advantage for in-liquid operation [118]. Figure 2.9 shows a photo of the electro-coil.

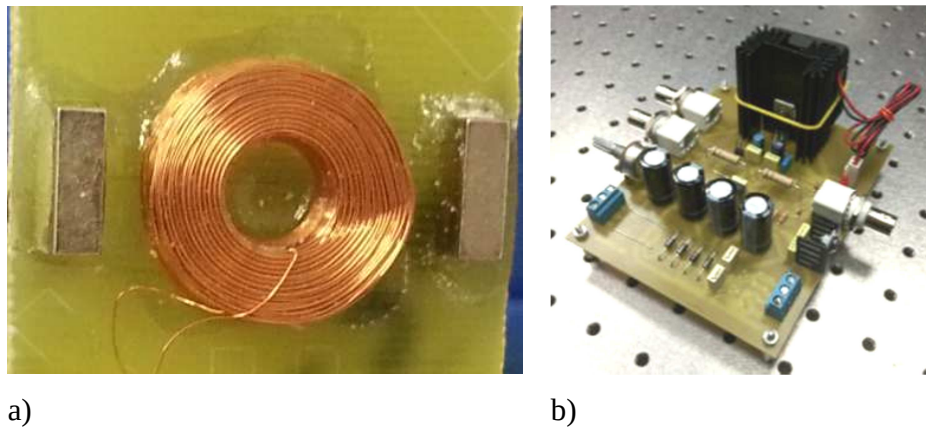


Figure 2.9. a) A photo of the electro-coil. b) A photo of the coil driver-circuit.

2.3.2 Optical Read-Out

Optical read-out enables remote sensing which does not require any electrical connections to the cantilever. This is a great advantage for point-of-care applications where disposable cartridges are needed. Different types of optical read-out schemes are tried within the scope of this thesis. An interferometric read-out based on embedded diffraction gratings is used for viscosity sensing applications. Other than that, commercial Laser Doppler Vibrometer (LDV) is used for further investigations on different applications.

2.3.2.1 Diffraction Grating based Read-out

A schematic view of the optical interferometric readout system is shown in Figure 2.10. An interference pattern is formed by laser beams reflecting from top Ni surface (movable cantilever) and bottom Si surface (fixed reference) (Figure 2.5b). This leads to an intensity modulation on the photodiode in response to the deflection of Ni cantilever with respect to the Si reference surface. Using the intensity modulation of the first diffraction order, the phase and frequency of the cantilever can be monitored [43, 44].

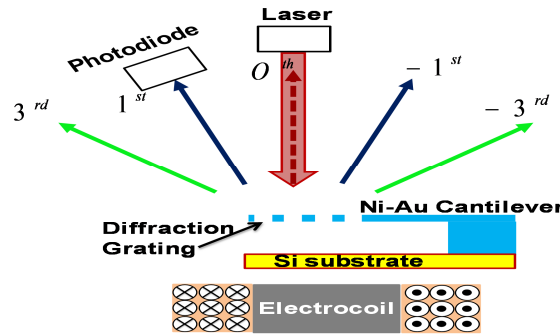


Figure 2.10. Schematic of the interferometric read-out. Optical interferometric read-out can be utilized with diffraction gratings at the tip of each cantilever [44].

Diffraction grating interferometry is an attractive method for micro-electro-mechanical-system (MEMS) devices to detect sub-nanometer displacements due to its high sensitivity with shot noise level detection capability [90, 119]. Optical readout using diffraction gratings provides sub-nanometer displacement resolution, which should bring a good mass sensing capability. The diffracted order intensities change sinusoidal with respect to the cantilever movement and can be expressed as [119]:

$$I_n \propto \sin^2\left(\frac{2\pi}{\lambda}g\right) \quad (28)$$

where n is n^{th} diffracted order light intensity for odd n , and g refers to the gap between cantilever and the underneath silicon bottom layer. The initial gap (g_0) is defined according to the etch depth and the cantilever residual bending due to the stress. For best responsivity,

the gap should be in the middle of the curve as shown in Figure 2.11. This results in half the maximum light intensity such that: $g_0 = \lambda/8 + k \cdot \lambda/4$ for any integer k .

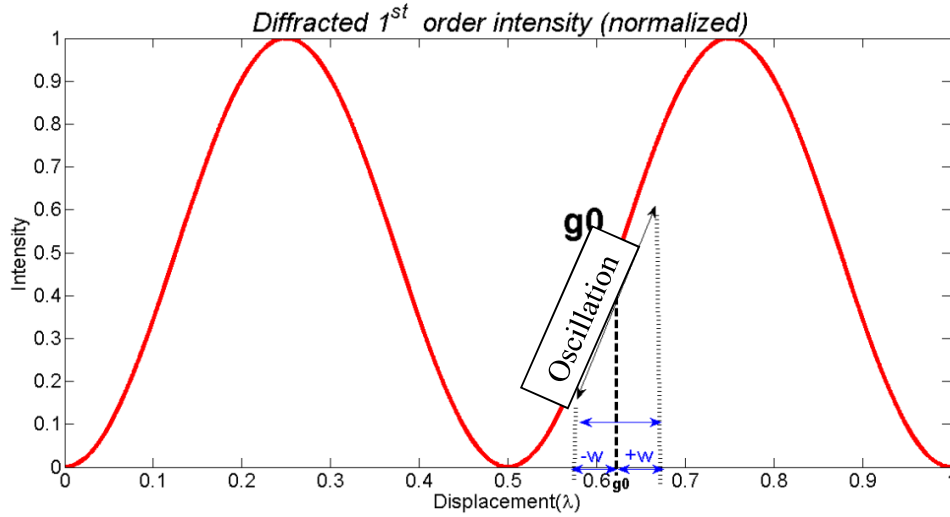


Figure 2.11. Diffracted order intensity vs. gap. In the ideal case where $g_0 = \lambda/8 + k \cdot \lambda/4$ intensity changes linearly with the oscillation.

The optical detection is carried out using a HITACHI 15 mW, 635 nm type laser diode, diffraction gratings embedded at the tip of each cantilever, a THORLABS PDA36A-EC type photodetector, and lenses. A photo of the optical setup is shown Figure 2.12.

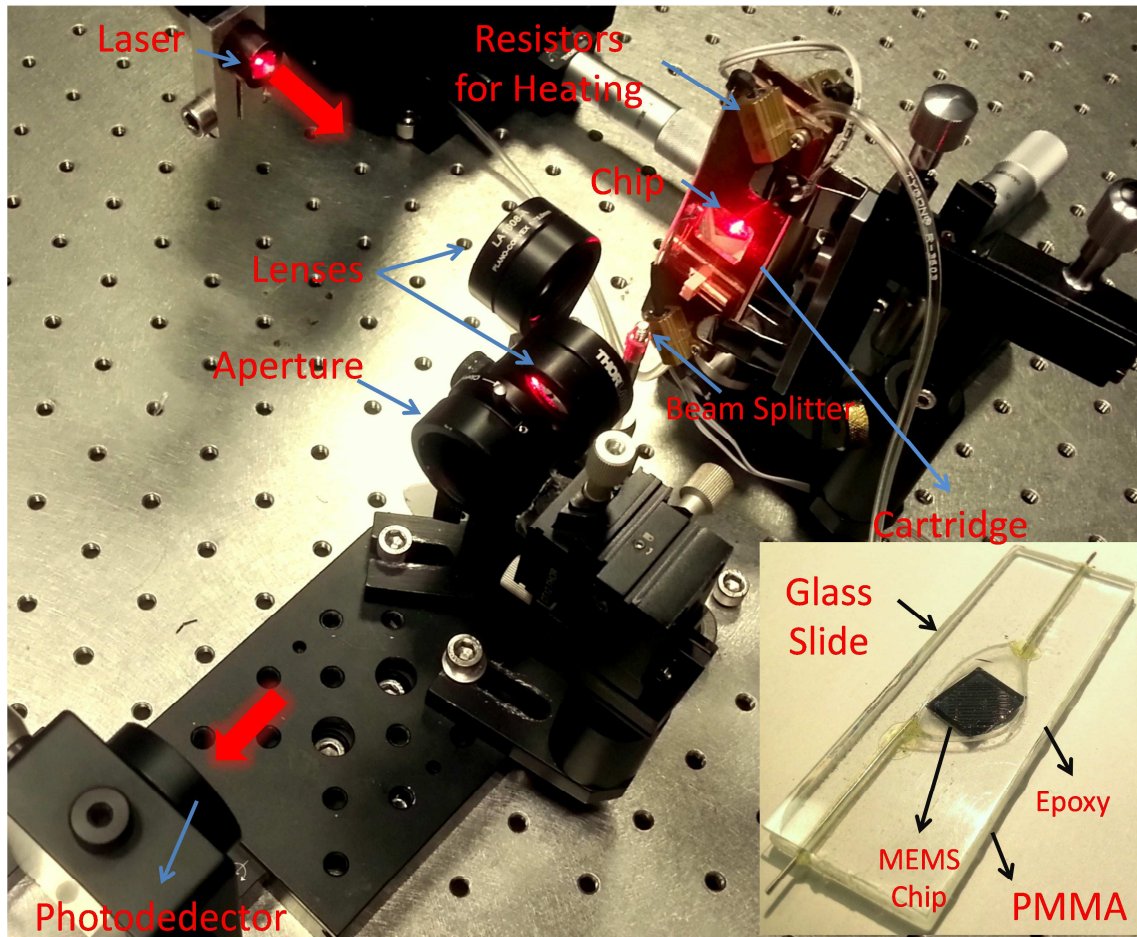


Figure 2.12: Picture of the measurement setup. The close-up picture of the PMMA flow cell is at the bottom right.

As seen in Figure 2.11 if the initial gap, g_0 location corresponds to the non-linear regions of intensity-displacement curve, this will result in higher harmonics in the oscillation waveform. Due to the silicon etching inhomogeneity and the intrinsic stress during electroplating and the gap g_0 becomes unpredictable and often away from the

desired value. This results in unpredictable non-linearity of the intensity read-out. Also there are some mechanical challenges related with the diffraction gratings. First of all the diffraction gratings at the tip of the cantilevers behaves like a tip mass. This results in lower frequencies and lower quality factors. Cantilevers also exhibit some static bending due to the intrinsic stress caused by the electroplating. When there are gratings at the tip of the cantilever, the stress induced bending increases due to heavier mass of the tip (Figure 2.5). In some experiments, the initial position (or DC position) of the cantilever may vary when different liquids are inserted. For instance, a dust particle or other loading at the tip can deflect the cantilever permanently and the position of the g_0 might change and the system starts behaving non-linearly. The Signal-to-Noise ratio is also very critical when trying to achieve high sensitivity for mass and viscosity detection. High SNR may be obtained with increasing the cantilever vibration amplitude by increasing the magnetic field applied. With the grating based system it gets challenging to detect the vibration amplitudes higher than $\lambda/8$ as the resultant intensity is not a monotonic function due to sinusoidal response curve [119].

In order to obtain higher sensitivity and higher Q for some applications the cantilevers without gratings are designed and used. A commercial Laser Doppler Vibrometer (LDV) is used to conduct the amplitude read-out with these cantilevers. The microscope image of the cantilevers with and without gratings are shown in Figure 2.13 a and b, respectively.

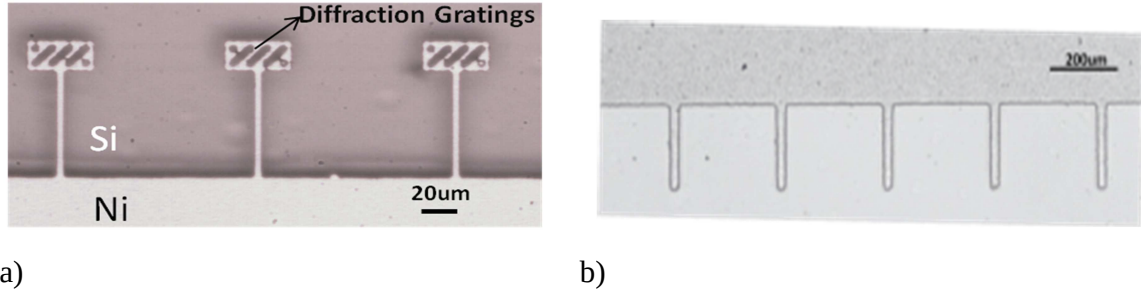


Figure 2.13. a) The cantilevers with diffraction grtings. b) The cantilevers without diffraction gratings.

2.3.2.2 Laser Doppler Vibrometer (LDV)

A laser Doppler Vibrometer (LDV) is a scientific instrument that is used to make non-contact vibration measurements of a surface [120]. The laser beam from the LDV is focused at the interested sample area, and the vibration amplitude and frequency are extracted from the Doppler shift of the reflected laser beam frequency due to the motion of the surface. The output of an LDV is generally a continuous analog voltage that is directly proportional to the target velocity component along the direction of the laser beam [121]. The LDV makes the vibration measurement without mass-loading the target, which is especially important for MEMS devices [122]. Laser Doppler Vibrometer is a very common equipment for measuring the vibration amplitude of the microcantilevers.

A vibrometer is usually a two beam laser interferometer that monitors the frequency (or phase) difference between an internal reference and a test beam. The test beam is sent to the target, and scattered light from the target is collected and interfered with the reference beam on a photodetector. Most commercial vibrometers work in a heterodyne regime by adding a known frequency shift (typically 30–40 MHz) to one of the beams. This frequency shift is usually generated by a Bragg cell, or acousto-optic modulator.

A schematic of a typical laser vibrometer is shown in Figure 2.14. The laser beam which has a frequency f_o , is divided into a reference beam and a test beam by using a beam splitter. The test beam then passes through a Bragg cell, in which frequency shift f_b is added to f_o . This frequency shifted beam then is focused on the target. The Doppler shift added to the beam due to the motion is given by $f_d = 2*v(t)*\cos(\alpha)/\lambda$, where $v(t)$ correspond to the velocity of the target as a function of time, α is the angle between the laser beam and the velocity vector, and λ is the wavelength of the light, which is 632 nm for red HeNe laser.

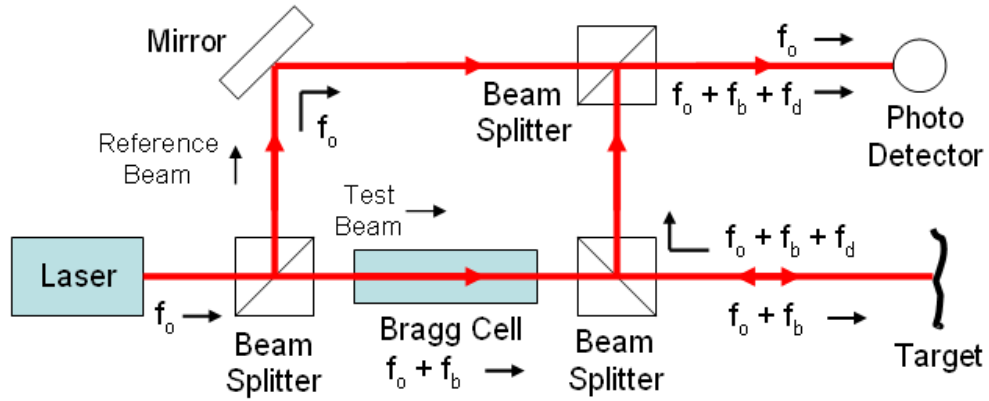


Figure 2.14. Basic Components of a Laser Doppler Vibrometer [120].

Light scattering from the target occurs in all directions. LDV collects some portion of the scattered light and reflected to the photodetector by the beam splitter. This light has a frequency equal to $f_o + f_b + f_d$. The combination of this scattered light and the reference beam is introduced to the photo-detector. The initial frequency of the laser is very high ($> 10^{14}$ Hz), which is higher than the response of the detector. The detector responds, however, to the beat frequency between the two beams, which is at $f_b + f_d$ (typically in the tens of MHz range) [120]. The output of the photodetector is a standard frequency modulated (FM) signal. The Bragg cell frequency is the carrier frequency, and the Doppler

shift is the modulation frequency. A demodulation step is utilized to derive the velocity, acceleration or displacement, time of the vibrating target.

A commercial Laser Doppler Vibrometer (Polytec: 3.4 MHz Laser Doppler Vibrometer) with a controller unit (OFV-5000 Modular Vibrometer Controller) is utilized for measuring cantilever frequency, amplitude and phase. Compared to optical interferometric read-out this commercial LDV is a mature measurement technique. This is an important factor, since the quality factors of the cantilevers are very low which results in a low Signal-to-Noise Ratio (SNR). Cantilevers vibrating at higher amplitudes can also be used with the LDV Setup. By using the LDV the effects of imperfections during the microfabrication is minimized. Hence more reliable and sensitive viscosity and mass measurements can be conducted and the systems' capabilities for different applications are investigated. For the product development steps the LDV can be altered with optical lever of fiber based read-out systems.

2.3.3 Electronic Readout: Frequency Tracking and Phase Tracking

Frequency sweeps are commonly used to find resonances however, this method is slow and cannot track the changes in real-time. There are two approaches in order to track the changes in cantilever dynamics real-time. The first one is to use a phase-locked loop (PLL) circuit and the second one is to use a lock-in amplifier. In the first approach, the system is driven in a closed loop manner at a fixed phase difference between the coil driver and the optical read-out signal. Whereas in the second approach, the cantilever is driven at a fixed frequency at or near its resonance and the phase shift in the detector output is monitored. Both the PLL based and the Lock-in Amplifier based approaches have been used throughout this research.

2.3.3.1 Frequency Tracking with PLL

The phase-locked loop (PLL) is a control system that produces a reference signal whose phase is related to that of the input signal [123]. The PLL is commonly used to excite a resonating system. For this kind of systems, there is a phase delay between the drive signal (produced by the PLL) and the system response. A change of the resonance frequency (for example as a result of the interaction between tip and sample as in AFM systems, or mass binding for biosensors) creates a change of the phase difference between the drive and the response. The PLL adjusts the drive signal frequency in such a way to restore the required phase delay.

A PLL is a feedback configuration having components: a phase detector (PD), a controller (commonly proportional-integral (PI), and a variable frequency oscillator. The comparison of the phase of the input signal to that of the reference signal derived from the oscillator is detected by the phase detector. A phase set point is dictated to the PLL. The phase difference must be made equal to that value: any deviation from the set point is the error signal. The PI controller and the oscillator are utilized to minimize this error signal. The system is named as phase locked loop since the two signals are related by their phases.

Defining Θ being the measured phase error between reference and input signal, f_c the center frequency which is typically set to a value close to the operating frequency of the PLL, T_i the integral gain and k_p the proportional gain of the PI controller, then the reference frequency, f_r generated by the variable oscillator satisfies [123]:

$$f_r = f_c + k_p \left[\Theta \frac{1}{T_i} \int \Theta dt' \right] \quad (29)$$

The purpose of the PLL is to accurately track frequency changes. Being a feedback control system with gain (given by the PI), the PLL is susceptible to instability if the parameters are not chosen correctly.

Two different PLL systems used in this study depending on the application needs. The first one is a custom made analog PLL whereas the other one is a PLL module of a commercial lock-in amplifier (Zurich Instruments HF2 LI). The custom home made PLL is a straightforward and fairly low-cost device, however it faces with instabilities due to the lack of packaging resulting in temperature instabilities. This causes to frequency instabilities and worsen the noise performance compared to the commercial device. Nevertheless it is useful for the applications which don't require high frequency stability performance. A frequency counter (Tektronix FCA 3100) is utilized to monitor the frequency and to average tens of thousands of cycles within a few seconds, which allows sub-Hz frequency sensitivity even in liquids. The equipment is capable of measuring frequency with 12 digit resolution up to 300 MHz, and the time resolution is 50ps. The device is connected to LabVIEW (NI, 2011) interface via USB port in order to record real-time data. A custom Labview interface is developed which records the real-time frequency data in a common data format. As the quality factors of the cantilevers with diffraction gratings in liquids are very low (around 1.5), the damped resonance peak amplitude occurs around 60° phase difference (not at 90°) according to the simulation as shown in Figure 2.15. Thus the custom PLL system is designed to track the resonance frequency at 60° phase difference for achieving maximum readout amplitude and signal to noise ratio (SNR).

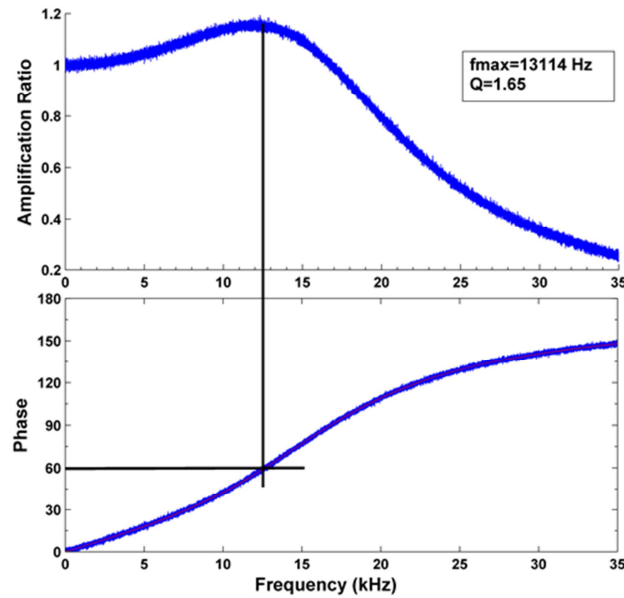


Figure 2.15. Simulated frequency response of the system using experimental values of $Q=1.65$ and $f_{\max}=13112\text{Hz}$ with added white noise. The cantilevers with diffraction gratings are used. The maximum amplitude is achieved at 60° phase difference which is the value that the system is locked by PLL.

The commercial HF2LI instrument provides a user interface in which the frequency sweeps and phase adjustments can be conducted. The real-time phase, frequency and amplitude read-out can be conducted by connecting this device to the Labview interface with a GPIB port. The frequency stability data obtained from different cantilevers and different PLL setups is shown in Figure 2.16. Both of the cantilevers are operating in DI water. It should be noted that the cantilever designs are also different; the first cantilever has the diffraction gratings at the tip whereas the other does not. The quality factor of the one corresponding to Figure 2.16a is around 1.5 whereas the corresponding to Figure 2.16b is around 3.

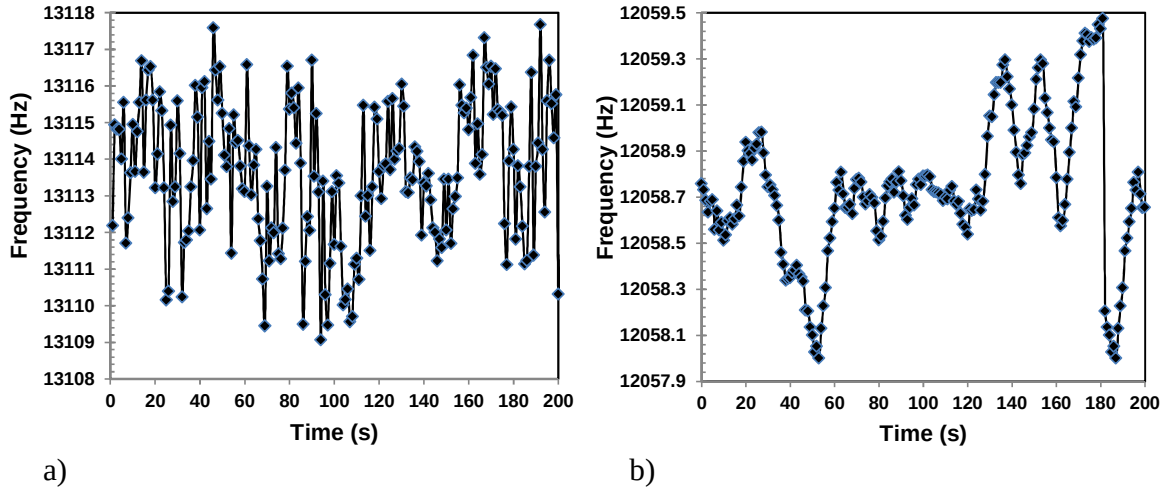


Figure 2.16 a) The frequency data obtained with the custom home-made PLL system. The average of 200 data points with 1 second intervals (200 sec.) is 13113.67 Hz and the standard deviation is 1.89 Hz. b) The frequency data obtained with commercial PLL based system. The average of 200 data points with 1 second intervals (200 sec.) is 13058.73 Hz and the standard deviation is 0.31 Hz.

2.3.3.2 Phase Tracking with Lock-in Amplifier

Lock-in amplifiers are capable of detection and measurement of very small AC signals—even down to a few nanovolts [124]. The device is often used to measure phase shift, even when the noise signals are high and there is low signal-to-noise ratio (SNR). They may make accurate signal measurements when the small signal is obscured by noise sources that are thousands of times larger. Lock-in amplifiers use the phase-sensitive detection (PSD) method to extract the component of the signal at a specific reference frequency and phase. Noise signals, at frequencies other than the reference frequency, are rejected and do not affect the measurement. Stated simply, PSD refers to the demodulation or rectification of

an AC signal by a circuit which is controlled by a reference waveform derived from the device which caused the signal to be modulated. The phase sensitive detector effectively responds to signals which are coherent (same frequency and phase) with the reference waveform and rejects all others.

A frequency reference is required for lock-in measurements. Usually the sensor is excited at a fixed frequency (from a function generator) and the response of the sensor at that reference frequency is detected by the lock-in amplifier. In Figure 2.17, the reference signal is a square wave at frequency ω_r . This might be the sync output from a function generator. If the sine output from the function generator is used for excitation, the response might be the signal waveform shown below. The signal is $V_{sig}\sin(\omega_r t + \varphi_{sig})$ where V_{sig} is the signal amplitude. The lock-in amplifier is capable of generating its own sine wave, shown as the lock-in reference below. The lock-in reference $V_L\sin(\omega_L t + \varphi_{ref})$. The lock-in amplifier amplifies the signal and then multiplies it by the lock-in reference using a phase-sensitive detector or multiplier. The output of the PSD is simply the product of the two sine waves given above:

$$\begin{aligned} & \frac{1}{2} V_{sig} V_L \cos([\omega_r - \omega_L]t + \varphi_{sig} - \varphi_{ref}) \\ & - \frac{1}{2} V_{sig} V_L \cos([\omega_r + \omega_L]t + \varphi_{sig} + \varphi_{ref}) \end{aligned} \quad (30)$$

The PSD output is two AC signals as shown in Equation (30), one at the difference frequency ($\omega_r - \omega_L$) and the other at the sum frequency ($\omega_r + \omega_L$). If the PSD output is passed through a low pass filter, the AC signals are removed and the output should be zero. However, if ω_r equals ω_L , the difference frequency component will be a DC signal. In this case, the filtered PSD output will be:

$$V_{PSD} = \frac{1}{2} V_{sig} V_L \cos(\varphi_{sig} - \varphi_{ref}) \quad (31)$$

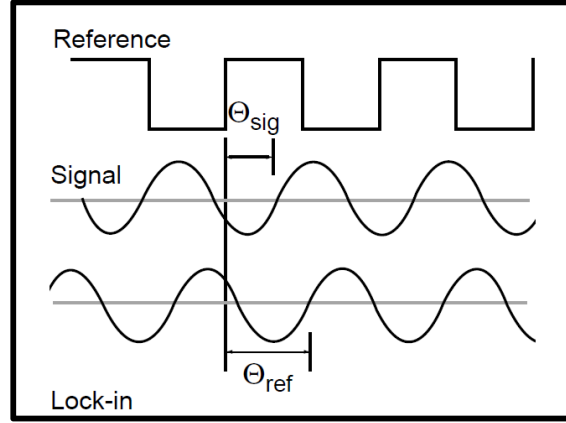


Figure 2.17. Diagram showing frequency reference, signal and Lock-in reference [124].

When the $\varphi = (\varphi_{sig} - \varphi_{ref})$ equals to 90° , there will be no output. This type of a lock-in with a single PSD is named as single-phase lock-in. The output of the first PSD is

$$V_{PSD1} = V_{sig} \cos \varphi.$$

It is possible to eliminate this phase dependency by a second PSD and making the system dual phase lock-in. If the second PSD multiplies the reference oscillator shifted by 90° ; $V_L \sin(\omega_L t + \vartheta_{ref} + 90^\circ)$. The output of the second PSD will be $V_{PSD2} = V_{sig} \sin \varphi$. There are two outputs existing. The phase between the signal and the lock-in is defined with:

$$\varphi = \tan^{-1} \left(\frac{V_{PSD2}}{V_{PSD1}} \right) \quad (32)$$

During the thesis in order to obtain meaningful signal with low SNR the lock-in amplifiers are utilized. The SR830 and the Zurich Instrument HF2LI are the most common state-of-the-art equipment having phase resolution, 0.01 and 0.001 respectively. In Figure 2.18a the amplitude and phase spectra of the same cantilever in different liquids is shown.

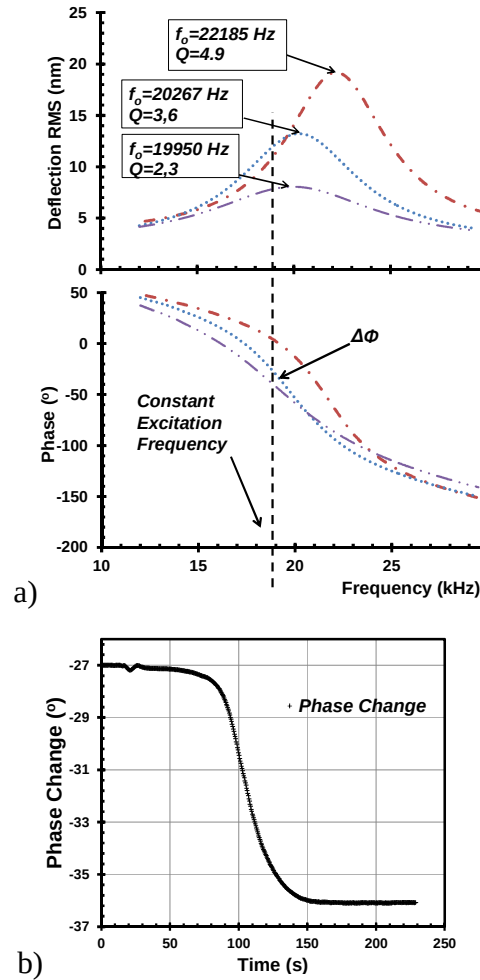


Figure 2.18. a) Example of amplitude and phase spectra of a cantilever in different liquids. The coil is driven at a certain frequency. The phase corresponding to this certain frequency changes in each liquid. By monitoring the phase the liquid properties can be extracted. b) The real-time monitoring of the phase during coagulation using Lock-in Amplifier. After the blood coagulates the phase corresponding to the constant frequency (about 18900 in (a)) shifts around 10° .

The vertical dotted line shows the phase value corresponding to a constant frequency for each curve. During the real-time measurement, we drive the coil at a constant frequency. When the liquid is changed or a reaction (coagulation or binding) occurs in the liquid, the phase value corresponding to that constant frequency changes. By using the lock-in amplifier this change can be monitored in real-time as shown in Figure 2.18b. The phase change depending on the viscosity, density and mass binding is monitored with the lock-in amplifier.

2.3.4 Temperature Controller Unit

Temperature is a critical parameter especially when operating in a viscous liquid media. The temperature change has an effect on viscosity and density of the fluid and elastic properties of the cantilever structure. The variation of these parameters results in a change of the resonant frequency. The change in viscosity of the fluid is the dominant parameter causing this frequency shift [125]. In order to limit temperature variations of the system an analog temperature controller system was added to the system. It consists of a temperature sensor, AD590, a controller circuit and heating units. Two different types of temperature stabilization units were used during this study. The sensor and the controller circuit were same in both of them. AD590 provides a linear temperature reading over its full range. At first four resistors were positioned symmetrically outside the flow cell (Figure 2.19.) for heating. Two copper layers were attached on the front and the back walls of the flow cell to conduct heat to the system in a uniform manner.

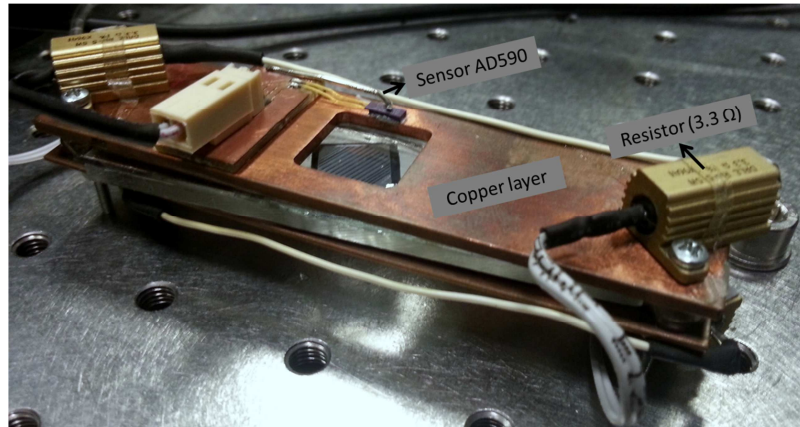


Figure 2.19. The copper temperature controller unit with heating resistors

Afterwards the copper layer was replaced with aluminum housing as shown in Figure 2.20. This aluminum block was designed using Solidworks software and afterwards machined with a CNC machine. In addition to temperature stabilization this aluminum housing enables a better fixing of the cartridge, easier assembly and handling. The cartridge could be fixed to the housing with the pins easily. Instead of large resistors, heating wires embedded to the aluminum block was used. This created a more homogeneous temperature distribution among the liquid in the cartridge. The temperature loss is less since the cartridge is buried inside aluminum block. Also the heating wires enabled faster heating compared to the resistors. During the applications in the earlier stages of this thesis the copper controller unit is used, for the later applications the aluminum housing is used.

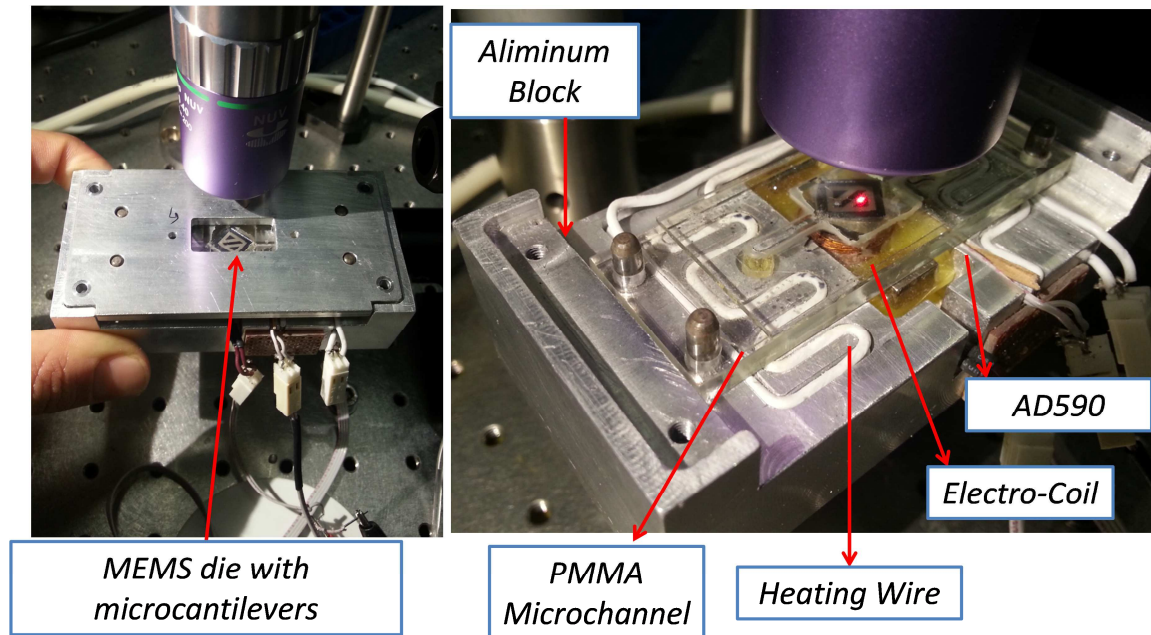


Figure 2.20. The aluminum temperature controller unit with heating wires.

2.4 Conclusion

The microfabrication scheme of the cantilevers is presented in this chapter. The components of the setups, which are responsible for actuation, sensing and data acquisition are also described in detail. Finally the theory of the cantilever dynamics including the in-liquid operation case is presented.

In the next chapters, the application of these techniques will be shown. The measurement setups and their components will be varied depending on the needs of the application. Also slight modifications in the microfabrication will be discussed. The viscosity of blood plasma and serum with high sensitivity is achieved by using these methods. Also the separation of the effects of viscosity and density is conducted with a novel method. Afterwards the application of the system for blood plasma coagulation times

will be shown in comparison with the commercial devices. Finally the system's capability for antigen detection in a multiplexed manner will be investigated.

3 BLOOD PLASMA VISCOSITY DETECTION

3.1 Introduction

Blood plasma viscosity can be used for the diagnosis of several diseases such as cardiovascular disorders, rheumatoid arthritis, certain autoimmune diseases [126]. Plasma viscosity increases in parallel with erythrocyte sedimentation rate (ESR); however, it is not influenced by hematocrit (anemia, polycythemia). Therefore, for hyperviscosity syndromes plasma viscosity can be more useful than ESR, which is widely used for diagnostics. The blood plasma viscosity range for healthy individuals is between 1.1-1.3 mPa.s. It can be elevated up to 3 mPa.s in symptoms of hyperviscosity [127]. Current devices for blood plasma viscosity measurement have limitations as reviewed below. This chapter reports a fast and convenient novel measurement method for measuring blood plasma and serum viscosity from low sample volumes with a high sensitivity.

There are several approaches for viscosity measurements. Viscometers based on the rolling ball (or falling sphere) principle have a wide usage for various liquids [103]. However, these instruments require custom-designed, precision-manufactured, long capillary tubes. These capillary tubes are costly and require cleaning after each use, thus not very suitable for home use. Another common viscometer is based on rotating tube principle [128] which is generally used as a bench top tool requiring high sample volumes. Capillary-tube viscometers are being used to measure the plasma or serum viscosity [129, 130] but suffer from cleaning requirement after each use which can be challenging due to the capillary geometry. Hence, there is a need for a viscometer which has disposable cartridges requiring low sample volume in order to facilitate blood plasma viscosity measurements in Point of Care (POC) tests.

The dynamic behavior of resonant microscale structures strongly depend on the medium in which they are operating [36]. Hence, this permits the measurement of the rheological properties of that medium by tracking the changes in resonant frequency and quality factors (Q). Different types of micro-mechanical viscosity sensors based on various techniques have been reported. These include, measurement of the torsional vibration of an oscillating tube, thickness shear mode resonators, acoustic wave sensors and oscillating plate/membrane structures [51, 57, 131-134]. Cantilever-based viscosity sensors also have a wide usage. Viscosity measurements of engine oils, silicone oils, ink, and glycerol solutions with the use of millimeter scale cantilevers with large sample volumes (around 3 ml) have been demonstrated [45, 55, 135]. Although there are other studies introducing measurements with microcantilevers [46, 50, 52-54, 86, 87], the working range or sensitivity is not suitable for the blood plasma viscosity measurement due to various limitations imposed by operating the cantilevers in liquids .

MEMS cantilever based viscosity sensors have been previously designed specifically for glucose concentration detection [81, 82, 84, 136]. Glucose levels are detected based on affinity binding of glucose sensitive polymeric matrix encapsulated over the sensor. Although these studies provide performance metrics of the sensor for various glucose solutions, the complete analysis of the sensor as a viscometer is not reported.

There are also commercial viscometers (Rheosense μ Visc [137] and Gluconline [138]) which utilize single-use cartridges and eliminate any requirements of cleaning. The sample is pumped inside a channel and the pressure difference along the channel is measured at two different locations by pressure sensors. The pressure drop between these two locations is used to calculate the viscosity of the sample. The primary downside of these systems is that they require bubble-free sampling or bubble trappers since bubbles cause additional pressure drop.

The microcantilevers and the sensing method developed in this thesis offer a novel method for fast and convenient blood plasma viscosity measurement with high resolution in a cartridge suitable for single use. In section 2.2, measurements with an interferometric optical read-out and PLL based frequency tracking setup are presented. The calibration and measurement protocol with that setup is described. The results of the measurements with Fetal Bovine Serum (FBS) and blood plasma samples are presented. The comparison of these results with commercial viscometers is also presented. In section 2.3, LDV and Lock-in amplifier based viscosity measurements are presented. The measurement protocol and the blood plasma viscosity measurement results are also given.

3.2 Viscosity Measurements with Interferometric Read-out & PLL

Real-time monitoring of cantilever resonant frequency is performed remotely using diffraction gratings fabricated at the tip of the dynamic cantilevers. The cantilevers have a length of 60 μm , excluding the head structure consisting of gratings, a width of 5 μm and a thickness of 1 μm . The diffraction gratings at the tip have 3 μm width, 20 μm length and the grating period is 6 μm . The close-up micrograph and the 3D drawing of the cantilevers can be seen in Figure 2.5 a.

After microfabrication, the chip containing microcantilevers is attached inside a microfluidic chamber by epoxy. The chamber is formed by machining 2 mm thick Poly Methyl Methacrylate (PMMA) plaque which is provided from local manufacturer. The channel depth is 500 μm and the total volume of the microchamber is 150 μl . The chamber is machined in water drop geometry (Figure 2.6) in order to prevent the sharp corners that cause bubbling during the liquid exchange. It should be noted that the sample volume requirement can be further reduced for a specific application. The chips are designed for proof of concept demonstrations. Hence, they have a large real estate (10mmx10mm) for ease of handling. The size of the chip can be reduced down to 1mm² and the required

sample volume can be reduced to 10 μl without much difficulty. The chamber has a single inlet and outlet with drilled holes for fluidic access. PMMA base is sealed by a standard glass slide using epoxy bonding (Figure 2.6). A photo of the measurement setup is shown in Figure 2.12.

The resonant frequency of the cantilever is tracked with the custom PLL control circuit as described in 2.3.3.1. The schematic of the measurement principle is shown in Figure 3.1. The viscosities of liquid samples are obtained through the measurement of the cantilever's frequency change with respect to a reference measurement taken within a liquid of known viscosity. We performed measurements with glycerol solutions at different temperatures and validated the repeatability of the system by comparing with a reference commercial viscometer. Experimental results are compared with the theoretical predictions based on Sader's theory described in section 2.1.2, and agreed reasonably well. The temperature is controlled with the copper temperature unit shown in Figure 2.19. Afterwards viscosities of different Fetal Bovine Serum and Bovine Serum Albumin mixtures are measured both at 23°C and 37°C, body temperature. Finally the viscosities of human blood plasma samples taken from healthy donors are measured.

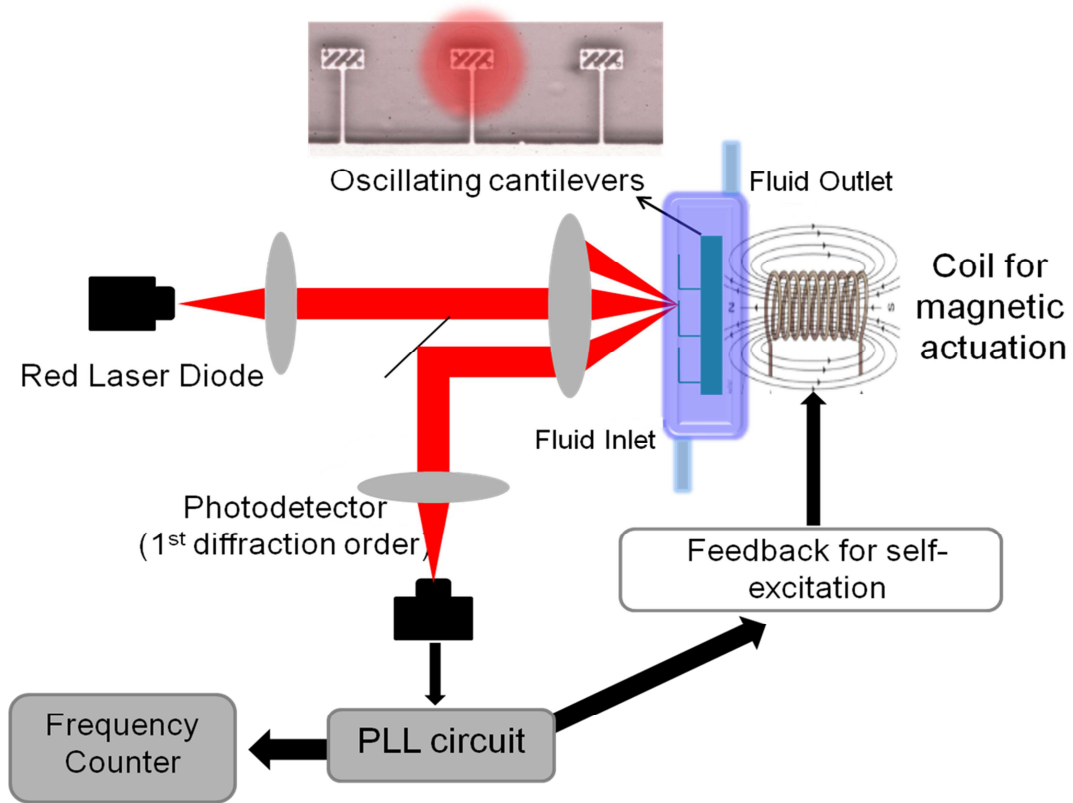


Figure 3.1: Schematic of measurement principle and setup. Actuation of nickel cantilevers is achieved with an external coil. Optical interferometric read-out can be utilized with diffraction gratings at the tip of each cantilever. The closed-loop system with phase-lock loop (PLL) circuit enables resonance frequency tracking.

3.2.1 Measurement Protocol

During the experiments deionized (DI) water at room temperature (23°C) is used as the reference liquid. Glycerol/DI solutions with varying glycerol concentrations have been used in order to calibrate the system and obtain its response to viscosity changes. The viscosities and densities of glycerol solutions used in the experiments can be seen in Table 3.1. In order to cover the viscosity range of blood plasma, glycerol solutions between 5%

and 40% are used which corresponds to viscosity range of 1.06 - 3.02 mPa.s. They are prepared with a glycerol solution having 86% concentration thus to determine the absolute glycerol concentration these values should be multiplied with 0.86. Viscosity values presented in Table 3.1 are obtained by the measurements with a reference commercial viscometer, Anton Paar AMVn [103]. This device works based on rolling ball principle: a gold-coated steel ball is introduced inside a glass capillary filled with sample. The time taken for the ball to roll a fixed distance between two magnetic sensors is converted into viscosity measurements.

Table 3.1: Viscosities and densities of each glycerol solution used in experiments. Viscosity values are measured using reference commercial viscometer, Anton Paar AMVn. Densities are volumetrically measured using a precision analytical balance (Precisa321LX).

Glycerol Percentage (%)	Viscosity (mPa.s)	Density (kg/m ³)
0	0.9532	0.9977
5	1.0642	1.0095
10	1.1698	1.0214
12	1.2323	1.0266
14	1.2685	1.0312
15	1.3040	1.0337
20	1.4838	1.0462
30	2.2843	1.0717
40	3.0221	1.0983

Measurement protocol is as follows:

STEP 1: Set the temperature to 23°C

STEP 2: Fill the microchannel with the DI water @ 23°C

STEP 3: Set the PLL phase to obtain maximum amplitude (Figure 2.15). This should be kept constant during the experiment

STEP 4: Record the frequency from frequency counter with a Labview interface.

STEP 5: Pause recording.

STEP 6: Drain the DI water

STEP 7: Introduce the sample liquid

STEP 8: Unpause recording

STEP 9: Record the frequency from frequency counter with a Labview interface **STEP**

10: Drain the sample liquid.

STEP 11: Fill the microchannel with DI water again

STEP 12: Repeat the STEPS 1 to 11 for each solution. First glycerol solutions with known viscosity and densities are used. Note the percent frequency changes for each solution.

STEP 13: A 2nd order polynomial is used for curve-fitting to the area of interest the (Figure 3.3) The fit function is used as a calibration curve for the subsequent measurements. The one time calibration for the cantilever is finished. This calibration is made since the geometries of the cantilever and its response to viscosity changes were unknown. This was necessary due to the microfabrication imperfections. When the fabrication is conducted at a MEMS foundry having exact recipes, this calibration step will not be necessary and the user may directly start from STEP 14.

STEP 14: Set the temperature to 23°C

STEP 15: Fill the microchannel with the DI water

STEP 16: Set the PLL phase to the same value.

STEP 17: Record the frequency from frequency counter with a Labview interface.

STEP 18: Pause recording.

STEP 19: Drain the DI water

STEP 20 Set the temperature to 37°C wait for 5 minutes to get stabilized

STEP 21: Introduce the blood plasma or serum sample at 37°C to microchannel

STEP 22: Unpause recording

STEP 23: Record the frequency from frequency counter with a Labview interface

STEP 24: Drain the plasma or serum sample

STEP 25: Set the temperature to 23 °C wait for 5 minutes to get stabilized

STEP 26: Fill the microchannel with the DI water

STEP 27: Calculate the percent phase change and determine the viscosity with using the calibration curve in Figure 3.3.

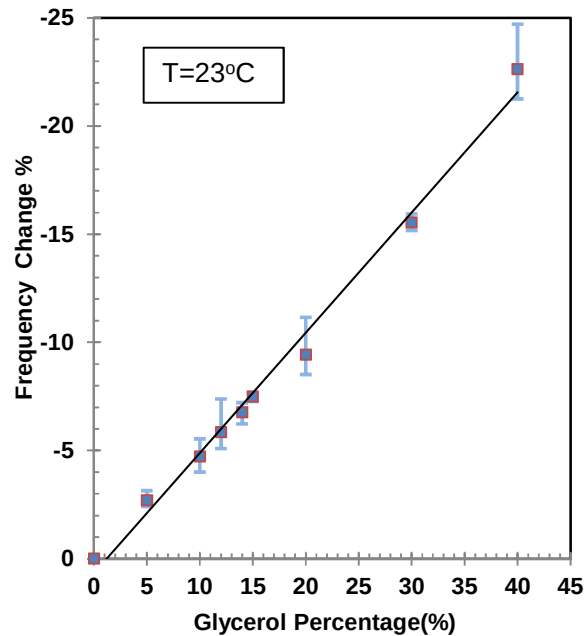


Figure 3.2. Percent frequency change obtained from different glycerol solutions at 23°C, room temperature. The data points show the averages of four different measurements for each glycerol solution (5%, 10%, 12%, 14%, 15%, 20%, 30%, and 40%). The error bars show the minimum and maximum values among these measurements. The line is the linear trendline fit to this data.

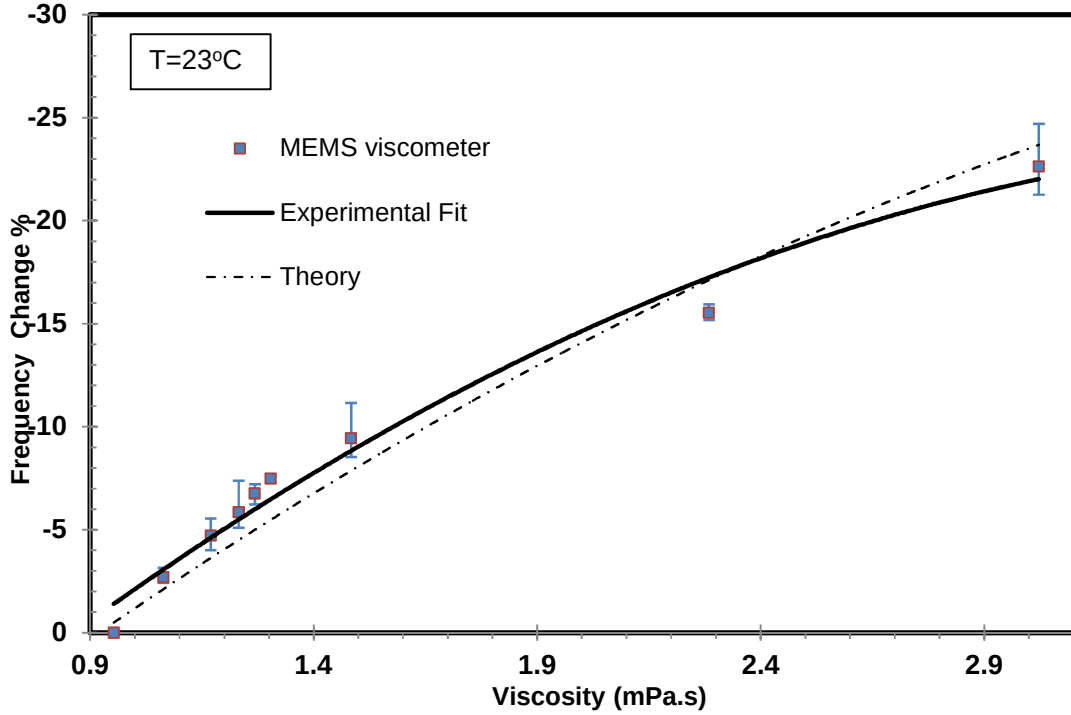


Figure 3.3. Percent frequency change in response to varying viscosity. The markers show the frequency change for each glycerol solution. The error bars show the minimum and maximum values of four measurements performed using different loadings of the sample cartridge. The bold line represents the 2nd order polynomial fit to the experimental data whereas the dashed line shows the results of the theoretical approach.

Viscosity values for each glycerol solution are obtained using Table 1 and the experimental frequency shift measurements are plotted in Figure 3.3. All of the MEMS viscosity measurement results in this paper are based on the experimental data and the fit function and the protocol described. In order to check the validity of the analytical formulas described in Chapter 2, theoretical predictions are also shown in Figure 3.3. According to equation(24) and (26) the relation between viscosity and frequency is quadratic. The slight

discrepancy can be associated with the assumptions used during the analytical approach [36] and experimental errors.

The variation between repetitive measurements of the same sample is also shown with the error bars. Although they are in an acceptable region, it can be asserted that experimental error during the liquid exchange and sample preparation and local heating of the sample with focused laser spot give rise to this variation.

The viscosities of the glycerol solutions with 10%, 12% 14% and 15% is prepared in order to observe the sensitivity of the system. The system is capable of resolving 0.04 mPa.s viscosity difference which is the difference between %14 and %15 glycerol solutions.

3.2.2 Results

3.2.2.1 *Measurements at different temperatures*

Increase in temperature results in a decrease in viscosity leading to an increase in the resonant frequency of the system. Also it should be noted that blood plasma and serum measurements should be conducted at 37°C body temperature, hence, reliability of the temperature control must also be confirmed. For this purpose viscosity of the 15% glycerol solution is measured at 23°C, 25°C, 30°C, 35°C and 40°C. The viscosity values obtained with MEMS viscometer and reference device are presented in Figure 3.4. The values taken with the MEMS viscometer is slightly smaller than the reference measurements (Table 3.2). This can be attributed to the influence of the local laser heating. The small volume around the cantilever can have slightly higher temperature than other portions due to laser heating. It is extremely challenging to measure local temperature variation in a given liquid at micro scale, therefore this hypothesis is not backed up with experimental data.

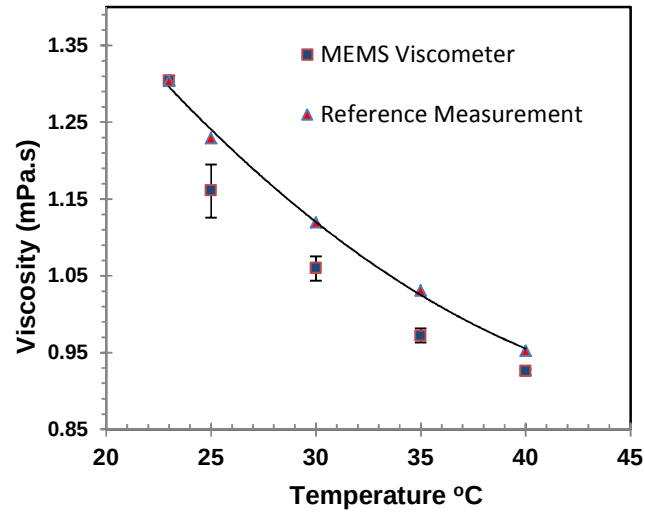


Figure 3.4. Frequency change due to temperature increase. Viscosities of 15% glycerol solutions at 23°C, 25°C, 30°C, 35°C and 40°C are measured with both reference commercial viscometer and proposed MEMS viscometer. Error bars represent standard deviation of 200 data points during one experiment. The error in the reference measurements are always lower than 1 μ mPa.s, hence they are not visible in the graph.

Table 3.2. Viscosities of 15% glycerol solution at different temperatures measured with both reference commercial viscometer and MEMS viscometer.

Temperature(°C)	Viscosity (mPa.s) Reference Measurement	σ (mPa.s)	Viscosity (mPa.s) MEMS viscometer	σ (mPa.s)	% difference
23	1.304	0.0008	1.303	0.006	-0.07
25	1.219	0.0010	1.161	0.033	4.75
30	1.119	0.0008	1.060	0.017	5.27
35	1.031	0.0011	0.972	0.009	5.72
40	0.952	0.0008	0.926	0.006	2.73

The mean value and the standard deviation (σ) reported in Table 3.2 and Figure 3.4 are computed using the 200 frequency measurements collected in 1 sec intervals. Frequency

counter can measure with $<1\text{mHz}$ accuracy while the standard deviation in the experimental data can be at 1-2 Hz level (Figure 2.16). According to Figure 3.3, the slope of the viscosity vs. frequency curve is in the range $[0.4-0.7] \times 10^{-3} \text{ mPa.s/Hz}$, therefore, the data is reported with 10^{-3} mPa.s accuracy, even though some data are noisier.

3.2.2.2 Measurements with FBS and BSA Solutions

Before measuring human plasma viscosity, viscosities of Fetal Bovine Serum, FBS (Sigma-Aldrich F2442, and Bovine Serum Albumin, BSA, (Sigma-Aldrich A3675) solutions were measured. By adding BSA into FBS the viscosity can be increased significantly whereas the density is not affected noticeably. This makes BSA-FBS solutions a good and easily accessible laboratory model of healthy/unhealthy human plasma. The hyperviscosity syndromes shows up around 1.8 mPa.s [127]. In order to cover that range, BSA-FBS solutions with different molarities are prepared. The molecular weight of BSA is 66.5 kDa . The viscosities of different solutions with 0M (only FBS), 0.66mM , 1.33mM , 2mM BSA were obtained from the MEMS viscometer both at 23°C (room temperature) and 37°C (body temperature) (Figure 3.5). The viscosity increase by the addition of BSA to FBS can be clearly seen.

Also the viscosities 0M (only FBS) and 2mM BSA obtained from the MEMS viscometer and reference commercial device are presented in Table 3.3. The values both at 23°C and 37°C are shown. The measurements show a good coherence with a small discrepancy, which can again be explained with the laser heating effect existing in MEMS viscometer.

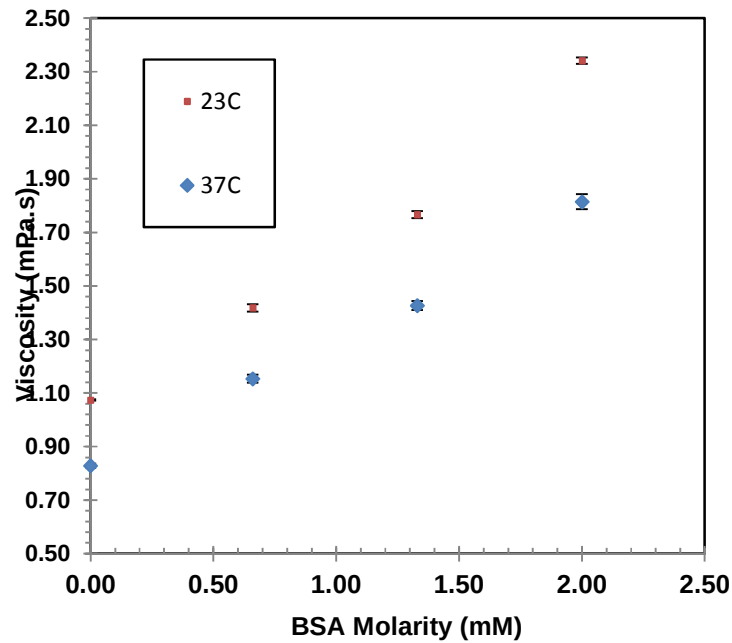


Figure 3.5. Viscosities of Bovine Serum Albumin (BSA) solutions (0M (only FBS), 0.66mM 1.33mM, 2mM) measured with MEMS viscometer. Measurements at room temperature and body temperature are shown. The error bars show the standard deviation of 200 data points during one experiment which can be named as the “noise floor” of the system.

Table 3.3. Viscosities of Serum and BSA solutions measured with both reference commercial viscometer and MEMS viscometer at room temperature and body temperature.

Solution Type	Temperature (°C)	Density (kg/m ³)	Viscosity (mPa.s) Reference Measurement	Viscosity (mPa.s) MEMS viscometer	% difference
FBS	23	0.906	1.105	1.075	2.7
FBS	37	0.906	0.861	0.828	3.8
2mM BSA	23	1.011	2.533	2.341	7.6
2mM BSA	37	1.011	1.808	1.815	0.4

3.2.2.3 *Measurements with Human Blood Plasma*

After the reliability of the system was checked using glycerol and serum measurements, measurements are conducted on plasma taken from healthy donors. After draining blood from each donor in a citrated vacutainer tube, the samples were spun at 5000 rpm for 10 minutes in order to separate plasma. The blood plasma viscosities of three different male donors obtained with MEMS viscometer are presented in Table 3.4. None of the donors has any known diseases showing hyperviscosity symptoms. Their plasma viscosities vary within the range of 1.1 mPa.s and 1.3 mPa.s, which is the normal blood plasma viscosity range [127]. Standard deviation of the measurement is also shown in Table 3.4. This value is noticeably higher than the deviations in glycerol measurements and FBS-BSA solutions. This situation is related with the relatively crude and bubbly nature of human blood plasma. It causes local variations of fluid property around the microcantilever and additional noise in optical read-out. Nevertheless, according to these results the system is capable of measuring plasma viscosity with a precision better than 0.03 mPa.s. It can be claimed that the device is suitable for screening of patients with hyperviscosity symptoms, having plasma viscosities above 1.8 mPa.s.

Blood plasma samples can leave hazardous residues inside the capillary tube of the reference device although it is rinsed after use. Hence the measurements with human blood plasma could not be repeated with the reference viscometer due to this contamination risk. This is a further proof that single use and disposable devices are important for measuring viscosity of biological fluids.

Table 3.4. Viscosities of Human Blood Plasma Samples taken from healthy donors. The measurements are conducted with proposed MEMS viscometer at body temperature (37°C). 'σ' is the Standard Deviation of 200 data points taken during the measurement.

Donor #	Viscosity (mPa.s)	σ (mPa.s)
1	1.280	0.017
2	1.257	0.026
3	1.183	0.014

3.2.3 Grating Readout Viscometer Conclusions

A protocol for measuring viscosity with our cantilevers is developed. The cantilevers with gratings are used and the read-out is conducted with the interferometric read-out method (Figure 3.1). The frequency is tracked with the custom PLL (described in 2.3.3.1). The proposed system is capable of measuring 0.04mPa.s viscosity difference between 0.86 and 3.02 mPa.s which covers the viscosity range of blood plasma. The effect of temperature on viscosity is investigated not only with the MEMS viscometer but also with a reference commercial viscometer. It is seen that the proposed method gives very similar results with the reference measurements with a difference less than 6% in repeated trials at different times. Measurements with biological liquids are also conducted. The FBS and BSA solutions having viscosities varying between 0.86 mPa.s and 2.53 mPa.s are used. The viscosities of human blood plasma samples taken from healthy donors are measured. The standard deviation in these measurements is lower than 0.03 mPa.s. It can be claimed that the MEMS-based viscometer is capable of measuring blood plasma viscosity with high precision and detecting diseases with hyperviscosity symptoms. The system can also be utilized for blood plasma coagulation time measurements, which can be defined as a rapid change of plasma viscosity.

In addition multiplexed measurements can be easily achieved due to the remote nature of actuation and sensing. With the optical readout method of the system, it is possible to perform the readout of multiple sensors simultaneously using an array of optical detectors. Using the proposed method and by combining viscosity and clot time measurements with various agents, a hand-held commercial device with disposable cartridges can be developed.

3.3 Viscosity Measurements with LDV & Lock-in Amplifier

The sensing is conducted using a laser and facilitated by Doppler effect using Laser Doppler Vibrometer (LDV) as described in section 2.3.2.2. Instead of the PLL a lock-in amplifier (SR 830) is used and the phase difference is tracked (as described in 2.3.3.2). In this section also the cantilevers doesn't have the diffraction gratings and they have different geometries (Figure 2.13b). The cantilevers have a length of 200 μm , a width of 20 μm and a thickness of 2.5 μm . Since the cantilevers are larger and thicker compared to the ones with diffraction gratings they have a better immunity to viscous damping which enables measurements of higher viscosities. With this method the sensor's viscosity resolution enhanced to 0.01 mPa.s in a range of 0.8 - 7.19 mPa.s.

3.3.1 Measurement Protocol

During the experiments deionized (DI) water at 30°C is used as the reference liquid. The protocol is similar to frequency tracking however this time, the phase changes are recorded instead of frequency.

The measurement protocol is as follows:

- **STEP 1:** Set the temperature to 30°C and wait approximately 5 minutes to get stabilized with 0.1°C precision

- **STEP 2:** Fill the microchannel with the DI water which was heated to 30°C in an external heater.
- **STEP 3:** Make a frequency sweep. Note the peak frequency.
- **STEP 4:** Drive the coil at that frequency with 1A current. This frequency will never change during experiments.
- **STEP 5:** Record the phase difference between the coil input and LDV output for 200 seconds
- **STEP 6:** Pause recording.
- **STEP 7:** Drain the DI water
- **STEP 8:** Introduce the sample liquid at 30°C to microchannel
- **STEP 9:** Unpause recording
- **STEP 10:** Record the phase difference between the coil input and LDV output for 200 seconds
- **STEP 11:** Drain the sample liquid.
- **STEP 12:** Fill the microchannel with DI water which was heated to 30°C in an external heater.
- **STEP 13:** Repeat the STEPS 1 to12 for each solution. First glycerol solutions with known viscosity and densities are used. The real time phase changes with respect to DI water for each glycerol solution are shown in Figure 3.7.
- **STEP 14:** A 2nd order polynomial is used for curve-fitting to the area of interest the (Figure 3.9) The fit function is used as a calibration curve for the subsequent measurements. The one time for the cantilever is finished. This calibration is made since the geometries of the cantilever and its response to viscosity changes were unknown. This was necessary due to the microfabrication imperfections. When the fabrication is conducted at a MEMS foundry having exact recipes, this calibration step will not be necessary and the

user may directly start from STEP 15. The frequency that the coil will be driven will be dictated by a barcode.

- **STEP 15:** Set the temperature to 30°C and wait approximately 5 minutes to get stabilized with 0.1°C precision
- **STEP 16:** Fill the microchannel with the DI water which was heated to 30°C in an external heater.
- **STEP 17:** Drive the coil at the dictated frequency with 1A current.
- **STEP 18:** Record the phase difference between the coil input and LDV output for 200 seconds
- **STEP 19:** Pause recording.
- **STEP 20:** Drain the DI water
- **STEP 21:** Set the temperature to 37°C wait for 5 minutes to get stabilized
- **STEP 22:** Introduce the blood plasma sample at 37°C to microchannel
- **STEP 23:** Unpause recording
- **STEP 24:** Record the phase difference between the coil input and LDV output for 200 seconds
- **STEP 25:** Drain the plasma sample
- **STEP 26:** Set the temperature to 30°C
- **STEP 27:** Fill the microchannel with DI water which was heated to 30°C in an external heater.
- **STEP 28:** Calculate the percent phase change and determine the viscosity with using the calibration curve in Figure 3.9

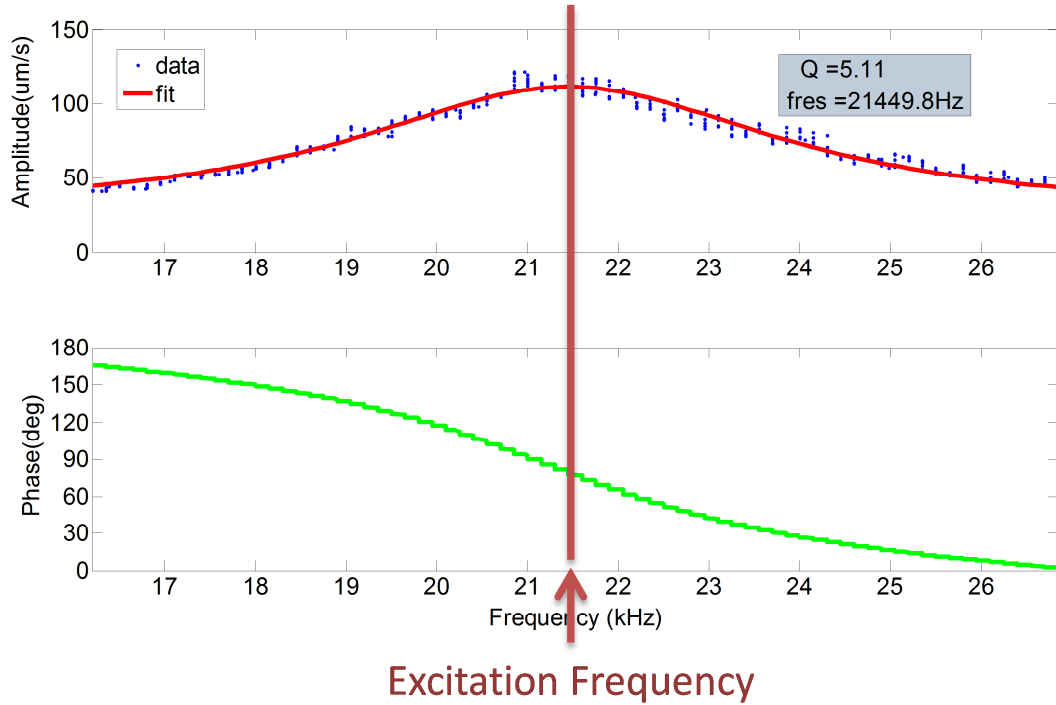


Figure 3.6: The amplitude and phase spectra of the cantilever in DI water at 30°C. The peak frequency is chosen to be the excitation frequency. The coil is driven at that frequency and real-time phase difference between the coil input and the LDV output is monitored using the lock-in amplifier (SR830)

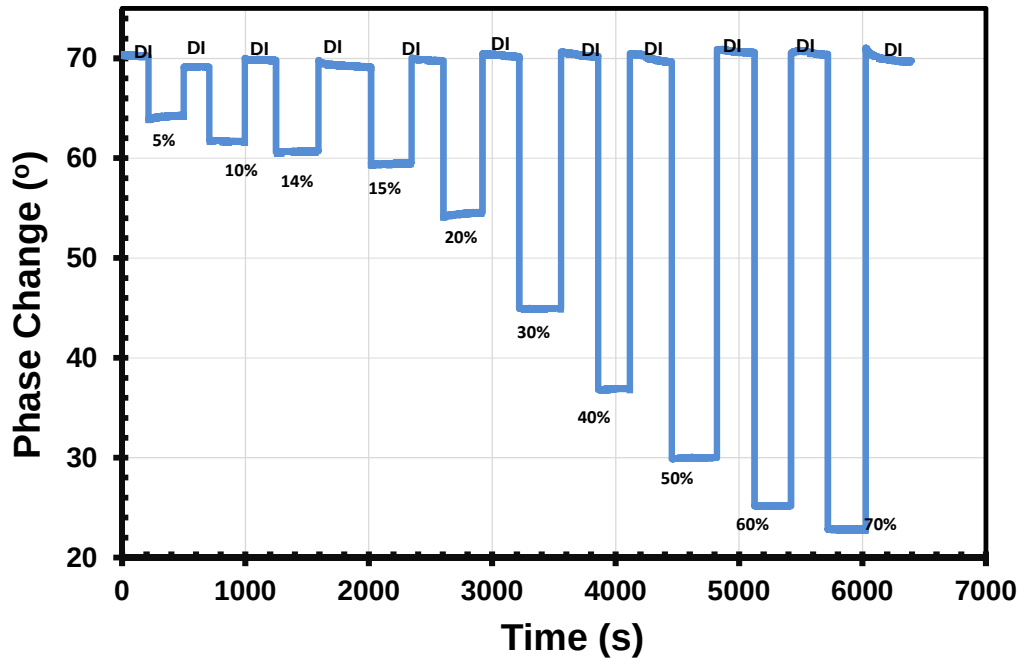


Figure 3.7: The real time monitoring of phase difference during the glycerol solutions and DI water exchanges.

The experiment is conducted at 30°C. Good repeatability is obtained with DI water after each step. The percentages of solutions used during the experiments are 5, 10, 14, 15, 20, 30, 40, 50, 60, and 70. They were prepared with a glycerol solution having 86% concentration thus to determine the absolute glycerol concentration these values should be multiplied with 0.86. The Aluminum temperature controller unit in Figure 2.20 is used.

The change of phase with respect to DI water for each glycerol solution is shown in Figure 3.7. After each glycerol solution the channel is filled with DI water again. As can be seen in Figure 3.7 the phase values at each repetition show a good coherence. The slight discrepancy can be related with experimental errors during liquid exchange. The standard deviation of the phase (for successive 100 data points) value in all liquids is less than 0.02°.

The system can clearly distinguish the difference between 14% and 15% glycerol solutions which corresponds to 0.04 mPa.s viscosity difference. The difference between the phases of these two solutions is 0.7° . When the 0.01° standard deviation is considered according to the phase stability data shown in Figure 3.8, it can be claimed that the system is capable of measuring viscosity with better than 0.001 mPa.s resolution in that region. Also the dynamic range is 0.8 to 7.2 mPa.s. Both the dynamic range and the resolution values are superior compared to the interferometric read-out and PLL based setup.

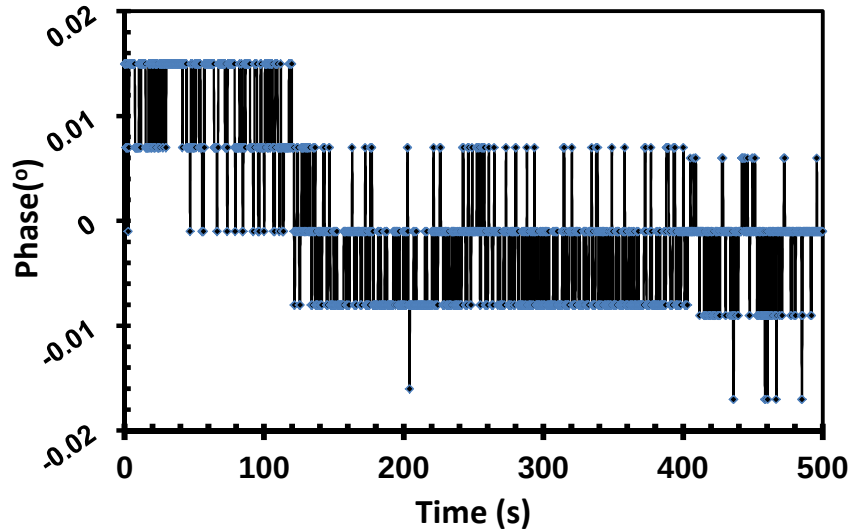


Figure 3.8: The phase stability data obtained in DI water at 21450 Hz. The standard deviation in 500 seconds is 0.007° . It is the quantization error which is bounded by the limits of the lock-in amplifier. The SR830 provides 0.01 phase resolution. The detection limit is defined by the lock-in amplifier.

3.3.2 Results

3.3.2.1 Blood Plasma Viscosity Measurements

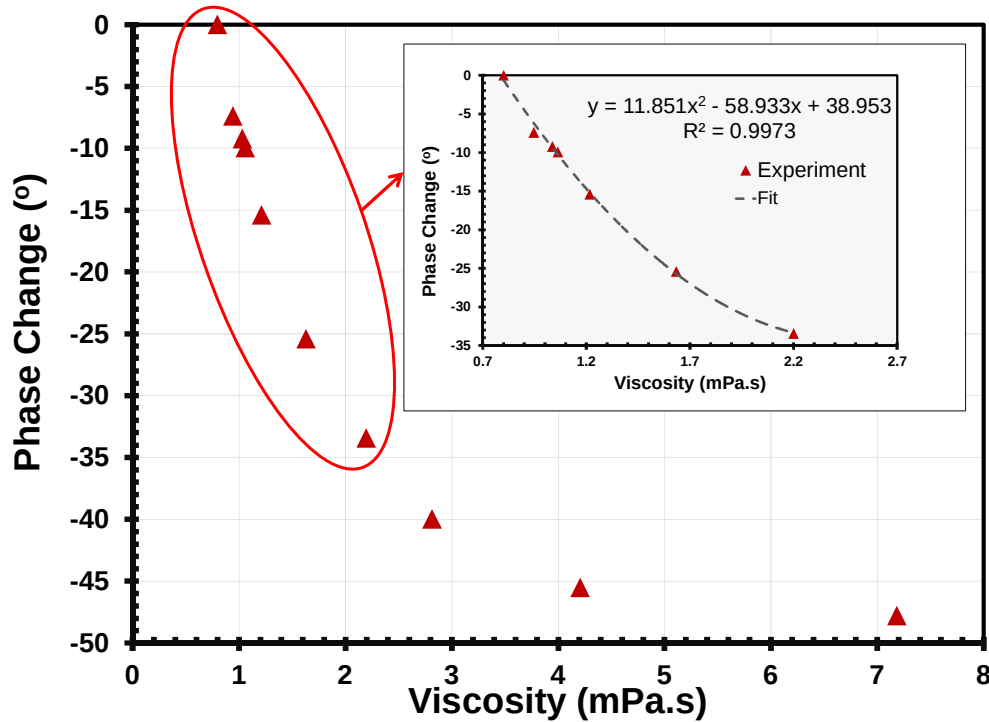


Figure 3.9: The change in phase in response to varying viscosity. The inset figure shows the 2nd order polynomial fit to experimental data in blood plasma viscosity region.

As mentioned before, blood plasma viscosities of healthy individuals vary between 1.1 mPa.s and 1.3 mPa.s. The hyperviscosity symptoms show up around 1.8 mPa.s and it can be elevated up to 3mPa.s [127]. The measurements are conducted with the samples taken from three healthy donors. The developed protocol is used to obtain viscosity values.

Table 1 shows the results of these measurements. As expected all the viscosity values are between 1.1 mPa.s and 1.3 mPa.s. Unfortunately we didn't have a chance to test the system with an unhealthy plasma sample due to high contamination risk in the laboratory

environment. Nevertheless, it is obvious that the system is capable of recognizing unhealthy blood plasma sample which generally shows up around 1.8 mPa.s.

Table I. Measured viscosities of blood plasma samples taken from healthy donors

Donor #	Viscosity (mPa.s)
1	1.132
2	1.227
3	1.242

3.3.3 Conclusion

A microcantilever based viscometer facilitating an LDV and a lock-in amplifier is developed. A measurement protocol is proposed which is very similar to the one using PLL. The system's viscosity sensitivity and dynamic range improved significantly compared to grating based read-out system. Fast and convenient measurement of blood plasma viscosity with a resolution better than 0.01 mPa.s is enabled with the proposed system. The system is capable of measuring viscosities between 0.8 mPa.s-7.2 mPa.s. Due to remote nature of actuation and sensing multiplexing is available for further applications.

New methods to measure the blood plasma viscosity with high precision are introduced using microcantilevers. Measurement protocols are built-up for two different setups. This MEMS-based viscometer enables measurement of blood plasma viscosity with a low sample volume requirement (150 μ l) and can be used in settings where disposable measurement units are a necessity. In the limit this volume can be further reduced to 10 μ l with appropriate chip design.

4 DENSITY AND VISCOSITY SEPARATION

4.1 Introduction

The ability to monitor viscosity and density with low sample volumes is important for various industrial and medical applications. Many industrial producers such as oil and ink use viscosity and density measurements for quality control purposes. In health care, blood viscosity is monitored for early diagnosis of diseases. The MEMS based sensors may provide versatile tools meeting the requirements of these vast applications. The microcantilevers are widely used for viscosity and density sensing applications due to their simple microfabrication [46, 48, 53-55, 69, 71, 73, 84, 85, 139, 140] as well as being well suited for applications where measurements from small volumes are required. The dynamic behavior of a cantilever changes depending on the viscosity and the density of the fluid medium it operates in [36]. Hence, by tracking the resonant frequency and the damping (Quality factor), significant data about the density and viscosity can be gathered. The effects of density and viscosity on both resonant frequency and quality factor are coupled [36, 46, 113]. Some groups worked on analytical methods to separately obtain the viscosity and density values from experimental data [46, 48, 53, 54]. Although the derived equations are applicable for a large viscosity range, they require precise vibration amplitude measurements, to obtain high accuracy. A general approach to separate viscosity and density effects experimentally is to relate quality factor changes with viscosity and resonant peak shifts with density [69, 71, 85, 140]. Ghatkesar et al. [71] obtained quality factors and resonant frequencies of cantilevers operating in aqueous solutions of glycerol and ethylene glycol. They utilized a network analyzer to record amplitude and phase spectra. Hur et al. [140] also recorded amplitude spectra to attain the resonant peaks and full width at half maximum (FWHM) to determine viscosity and density. With this approach in order to

attain high sensitivity in a broad dynamic range frequency sweeps with high frequency resolution is necessary. Rust et al. [85] used an empirical way to measure the viscosity and the density of different DNA solutions separately. They also relate the frequency shift with density and damping with viscosity. Instead of recording the full amplitude spectra they monitored the frequency at two different phase difference to extract the damping term. They achieved the measurement accuracy of 0.4% for density in 990-1050 kg/m³ range and 6% for viscosity for 0.94-2 mPa.s range. Bircher et al. [69] investigated the polymerization of the acrylamide by real time monitoring the viscosity and density. They adapted Sader's theory for higher modes to obtain higher sensitivity and accuracy. As in [71] they used network analyzer to gather high resolution amplitude and phase spectra with high speed. They reported the viscosity with 10 % accuracy and density with 4% accuracy for the ranges 1-10 mPa.s and 998 to 1176 kg/m³, respectively.

The frequency sweep approach is also used with other types of MEMS structures [64, 65, 95, 105]. Lee et al. [65] and Khan et al. [64] used suspended microchannel resonators (SMR) to measure the viscosity and density precisely in a limited range. Since the SMR type resonators have high quality factors they have inherent ability to maintain high Signal-to-Noise Ratio (SNR) and high resolution. However they have a significant microfabrication complexity compared to microcantilevers. In another recent work Kim et al. [95] facilitated piezoelectric layer embedded- microdiaphragm type resonator to measure viscosity and density of blood serum. As in the other references, they utilized amplitude spectrums to obtain the viscosity and density separately. Manzanque et al. [105] used a piezoelectric MEMS resonator . They measured the frequency and amplitude with a phase lock loop (PLL) circuit and applied the analytical formulas [54]. To the best of our knowledge, in the published literature both the frequency and the quality factor are used for separating the effect of viscosity and density. This approach is slow due to the requirement of high resolution frequency sweep.

In the previous chapter we utilized nickel cantilevers to measure viscosity of blood plasma and serum [141, 142]. We achieved an accuracy of 6% and a viscosity sensitivity of 0.01 mPa s whereas the density values were provided by external equipment. In this work we extend our previous techniques to simultaneous measurement of viscosity and density.

In this chapter, we introduce a novel empirical method for simultaneous measurement of viscosity and density by using cantilevers with different widths. The resonant frequency and quality factor are dependent on the hydrodynamic force acting on the cantilever. This hydrodynamic force is a coupled function of both viscosity and density. The effect of viscosity and density will differ depending on the cantilever geometries [53, 143]. We fabricated cantilever designs with varying widths while keeping the length and the thickness same. Calibration liquids with water solutions of ethylene glycol and glycerol were prepared [71, 85]. We use a commercial PLL to track the frequency at constant phase, record the frequency change in these different liquids with respect to DI water. For each cantilever design the same procedure is repeated and calibration constants are determined. After the calibration step, the viscosities and densities of different solutions are determined with the proposed method and the results are compared with reference measurements.

4.2 Materials and Methods

4.2.1 The Measurement Principle and Fabrication

Similar to the measurement principle in the previous chapter, the nickel cantilevers are subjected to an AC magnetic field provided by an external electro-coil. The read-out is conducted with a Laser Doppler Vibrometer within the scope of this chapter. The PLL module of the commercial lock-in amplifier (Zurich Instruments HF2LI) is utilized for frequency tracking as shown in Figure 4.1.

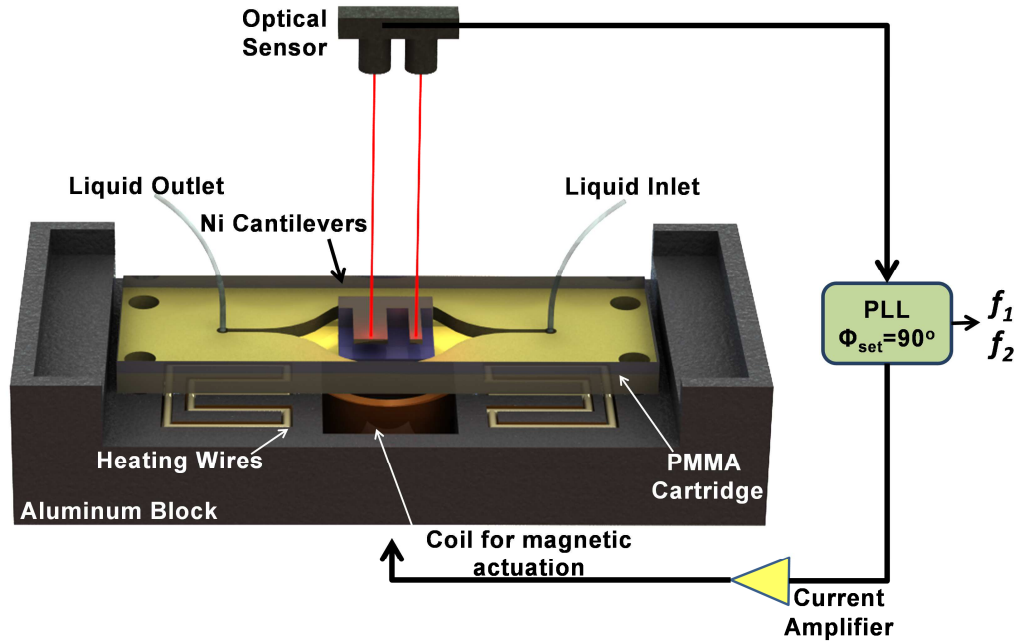


Figure 4.1 The schematic representation of the measurement principle. Actuation and sensing is conducted with an external electro-coil and Laser Doppler Vibrometer (LDV), respectively. Two cantilevers are immersed in the liquid and have different widths (25 μm and 100 μm) and the same thickness (1.75 μm) and length (200 μm).

The cantilevers are fabricated with the same one mask microfabrication process without any significant modification. The total etch depth is 80 μm , which is sufficient to avoid squeeze film damping effect during operation. The fabricated cantilevers have different widths (25 μm , 100 μm) and same thicknesses (1.75 μm) and they don't contain any diffraction grating. The lengths of the cantilevers are also same in the mask design (200 μm). However during anisotropic etching a certain undercut occurs for the cantilevers with 25 μm width, while waiting for the 100 μm to get released. This undercut value is also added to the theoretical calculations in the light of the frequency measurements in air.

After the chips are fabricated they are placed inside a PMMA channel. This channel consists of three different PMMA layers. The bottom layer houses the chip, channel geometry is patterned into the mid-layer by laser cutting and the top layer is used as a lid. The thickness of each layer is 1 mm. The circular geometry is required to obtain a perfect liquid exchange and avoid liquid residues from the previous sample. The chips are bonded to the bottom layer by double sided tape. Finally, all of the layers are bonded to each other by using chloroform. The 3D drawing of the cartridge layers are shown in Figure 4.2a. The sample volume required to make measurement is 100 μl and it can be further reduced with optimized chip and channel design. A photo of the final cartridge is shown in Figure 4.2b.

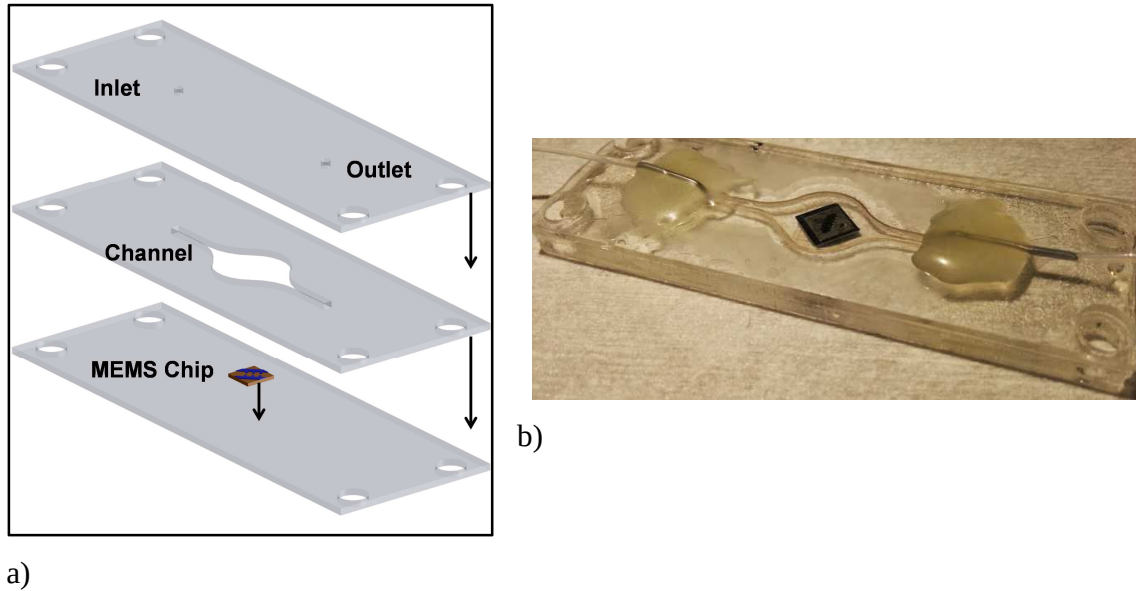


Figure 4.2.a) The 3D drawing of the PMMA cartridge layers. b) a photo of the PMMA cartridge

4.2.2 The Method

As can be seen from Equation (22) and (23) the ratio between the viscous layer, δ and the cantilever width, w is a critical parameter. The effect of viscosity and density on

resonant frequencies of cantilevers with different widths will significantly vary. According to this theory, when the density is constant the effect of viscosity change on the resonant frequency will be lower for wider cantilevers. Whereas for the constant viscosity case the added mass term will be dominant, which is highly dependent on the density changes. In the light of this assumption we define a frequency change as a function of viscosity, μ and density, ρ :

$$\Delta f_i(\mu, \rho) = a_i\mu + b_i\rho + C_i \quad (33)$$

The i indices show the cantilever design number associated with the width of the cantilever. The Δf represents the percent frequency change with respect to the frequency in DI water. The coefficients a_i , b_i and C_i are obtained from the calibration measurements using the special solutions with the same viscosity but different densities and the same density but different viscosities. Glycerol and Ethylene Glycol solutions are used to obtain these special values. The viscosities of these liquids were measured with Anton PAAR AMVn commercial viscometer[103] whereas the densities were measured using Anton PAAR DMA 4500 M density meter [104]. The viscosity and densities of these calibration liquids are shown in Table 4.1

Table 4.1: Densities and viscosities of the reference solutions for calibration experiments conducted at 23°C.

Solution	Density (kg/m^3)	Viscosity ($mPa.s$)
DI H ₂ O	997	0.935
G8.6 (8.6 % glycerol)	1021	1.175
E10 (10 % ethylene glycol)	1010	1.175
G5 (4.8% glycerol)	1010	1.060

The amplitude and phase spectrums of different cantilevers in these calibration liquids are shown in Figure 4.3. As expected the cantilever with 25 μm width has a lower Q, which

means that it is less immune to viscous damping. Also the pure density difference, which is the difference between the liquids G8.6 and E10, is more effective on 100 μm width cantilever. However it is not easy to observe these differences clearly and practically with a frequency sweep. It is clear that to distinguish the resonant peaks a frequency sweep with very high frequency resolution is required [69, 71]. Furthermore, calculating the Q-factor accurately requires a frequency sweep covering a broad range due to large damping.

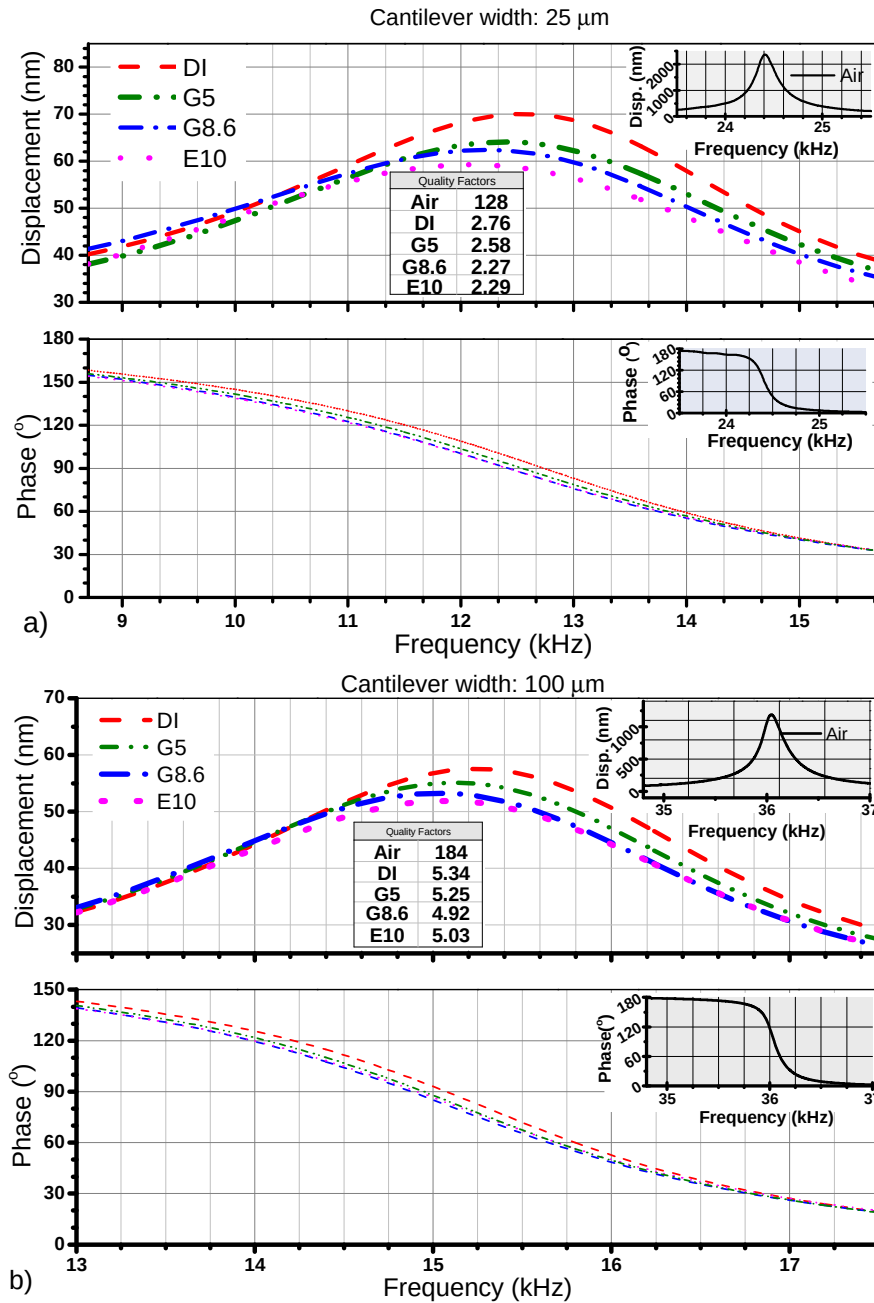


Figure 4.3: Amplitude and Phase Spectrums of cantilevers with 25 μm (a) and 100 μm (b) widths in air, DI water, G5, G8.6 and E10. PLL is set to 90° phase difference.

In this section, instead of conducting high resolution frequency sweeps we tracked the frequency at a certain phase with the PLL. The phase of the PLL was set to 90^0 for all the cantilevers. The experimental procedure is as follows; at first we introduced DI water in the microfluidic channel and recorded the frequency at 90^0 phase difference. After taking data for 200 seconds, the DI water was drained and the next sample was introduced into the channel. The frequency was again recorded for 200 seconds. In the last 100 second of this period the frequency got stabilized and the standard deviation of the frequency was less than 1 Hz for all cantilevers, which defines the noise floor of the system. Subsequently the sample was drained and DI water was introduced again. The data monitoring interval (200 seconds) can be reduced with more optimized PLL parameters for specific viscosity and density intervals. This procedure is repeated two times for each calibration solution. A real time plot of one experiment is shown in Figure 4.4. The difference between the frequency in DI water and frequency in the sample liquid is divided to the frequency in DI water and the percent frequency change is calculated. The same procedure is repeated three times for each cantilever design (50 μm , 100 μm widths) and the calibration curves are obtained. All the experiments are conducted at $23 \pm 0.1^\circ\text{C}$.

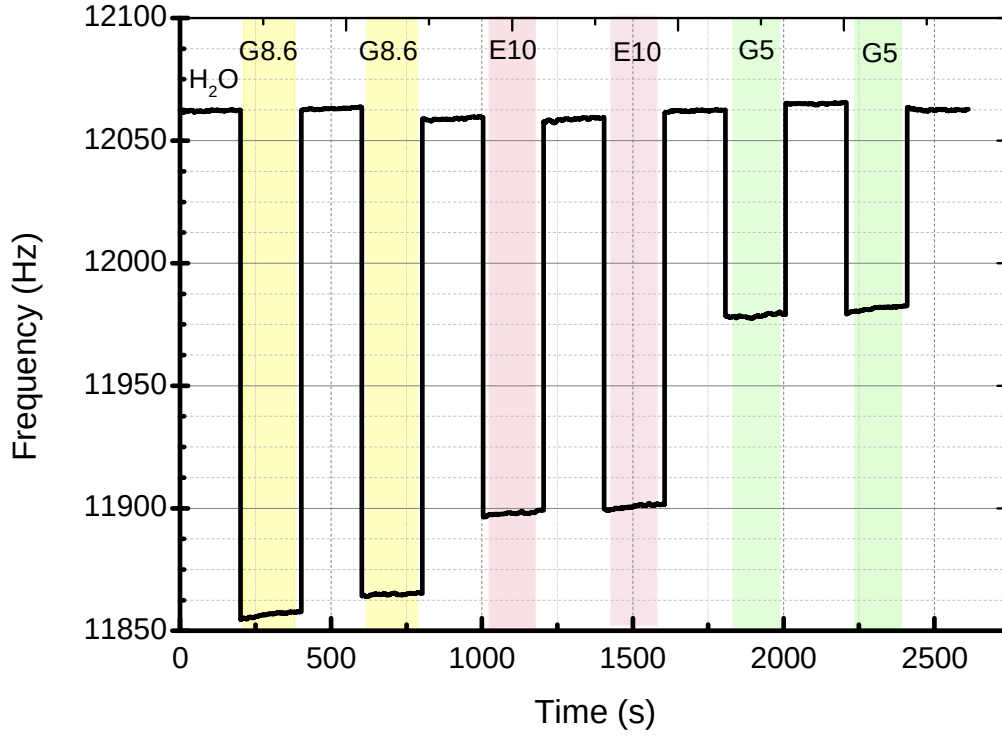


Figure 4.4: The real time plot of a calibration experiment. The frequency in DI water is 12060 Hz. The absolute frequency changes for G8.6, E10 and G5 are 200 Hz, 159 Hz and 83 Hz. The standard deviations of the frequencies at the last 100 s are less than 0.65 Hz for every liquid. The a_1 , a_2 , b_1 , b_2 and C_1 , C_2 values are 0.016, 0.033, 7.741, 3.409 and 23.321, 36.119, respectively.

By using the viscosity and density values for the calibration liquids shown in Table 1 and conducting the experiments for cantilevers with different width, the coefficients a_i , b_i and C_i in Eq. 7 can be determined. Then the frequency changes of the two cantilevers with respect to the frequencies in DI are measured (Δf_1 and Δf_2) and inserted into the Eq.8 below to solve for the unknown viscosity and density (μ, ρ) values:

$$\begin{Bmatrix} \mu \\ \rho \end{Bmatrix} = \begin{bmatrix} a_1 & b_1 \\ a_2 & b_2 \end{bmatrix}^{-1} \begin{Bmatrix} \Delta f_1 - C_1 \\ \Delta f_2 - C_2 \end{Bmatrix} \quad (34)$$

4.3 Results

As shown in Eqs (33) and (34), the approach is based on the linear relation between viscosity and frequency change. When the viscosity is further increased a quadratic relation occurs. Hence we added a μ^2 term to the right hand side of Eq. 7 and used a quadratic fit to achieve the new coefficients. For the viscosity range 0.6 to 2 mPa.s the linear assumption is used, whereas for the 2 mPa.s-10 mPa.s range the quadratic assumption is used. At first we tested our system with two cantilevers (with widths 25 μm and 100 μm). A third cantilever with 50 μm is also calibrated and added to the matrix for further improvement in accuracy and reliability.

To investigate the accuracy of the proposed method, water glycerol solutions G6 (6%), G13 (13%), G17 (17%), G25 (25%), G43 (43%) and G60 (60%) and water ethylene glycol solution E6 (6%) are prepared. All the experiments are conducted at 23 ± 0.1 °C. Also DI water at 35 ± 0.1 °C is used as a test liquid. Figure 4.5 shows the graphical comparison of the results obtained with the proposed method using two cantilevers (25 μm and 100 μm) and reference measurements.

Table 4.2 and Table 4.3 show all the results, standard deviations and the accuracy of the proposed method for density and viscosity, respectively. Using three cantilevers (25 μm , 50 μm and 100 μm) instead of two (25 μm and 100 μm) slightly improves the standard deviations which correspond to higher reliability. The accuracy of the density measurements is better than 3% for the density range 997 to 1150 kg/m³ which is comparable with the recent literature with the microcantilever based sensors [69] and other MEMS devices [105] based of frequency sweep techniques. The accuracy of the viscosity measurements is better than 5% for 0.935 to 4 mPa.s range which is comparable [69, 105] or better than [85] the published literature. The geometrical imperfections due to microfabrication (undercut and residual stress during electroplating), liquid exchange errors and local temperature instabilities due to laser heating are the main error sources. These

errors can be reduced with further improvements on measurement setup and microfabrication processes. Another important point is when the viscosity is further increased to 9.99 mPa.s the accuracy in viscosity measurements decreases significantly. This can be explained with the dramatic change in the phase curves for the high viscosity liquids which results in a saturation [141] . The minimum detectable viscosity and density change can be calculated from the noise floor of the frequency measurement (0.65 Hz) and the frequency difference between calibration liquids. Based on the signal-to-noise ratio, the minimum detectable difference in the viscosity is 1.6×10^{-3} mPa.s and the density is 0.3 kg/m^3 . Also when the minimum detectable frequency in theory is considered, the equation **(20)** is used and the values can be chosen for 100 μm width cantilever. By that assumption when the measurement bandwidth is chosen as 100 Hz due to the PLL bandwidth. The minimum detectable frequency can be calculated as 0.4 Hz. This can be considered as the theoretical noise limit.

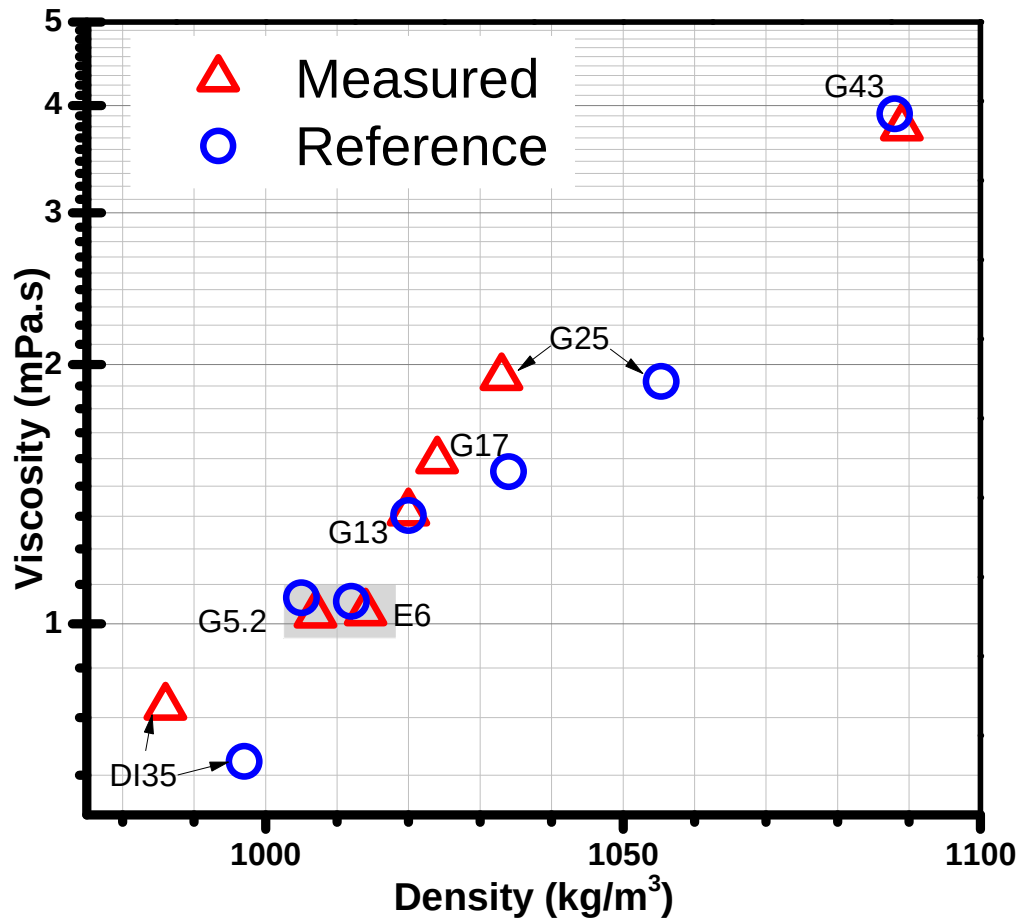


Figure 4.5: The graphical comparison of measured and reference values of test liquids. The reference viscosity measurements are conducted with Anton Paar AMVn capillary viscometer. The reference density measurements of DI35, G5.2, and E6 are conducted with Anton Paar DMA 4500 M density meter which provides a standard deviation less than $1 \times 10^{-1} \text{ kg/m}^3$. The reference density measurements of other solutions are conducted with high precision balance and micropipettes.

Table 4.2. Density results obtained from reference measurements and the proposed method. The standard deviation of 6 different measurements is shown. Error is the percent difference between the reference and measured values

DENSITY	Reference	2 Cantilevers		3 Cantilevers	
Liquid	<i>Mean (Standard Deviation) [kg/m³]</i>	<i>Mean (Standard Deviation) [kg/m³]</i>	<i>Error (%)</i>	<i>Mean (Standard Deviation) [kg/m³]</i>	<i>Error (%)</i>
DI35	994	986 (0.3)	0.8	995 (0.6)	0.1
G5.2	1012	1014 (0.5)	0.2	1016 (0.8)	0.4
E6	1005	1007 (0.6)	0.2	1011 (0.4)	0.6
G13	1020 (13.8)	1020 (6.8)	0.0	1017 (4.5)	0.3
G17	1034 (23.4)	1024 (6.1)	0.9	1018 (4.4)	1.5
G25	1055 (11.2)	1033 (13.3)	2.1	1026 (6.0)	2.8
G43	1088 (14.3)	1089 (9.5)	0.1	1086 (8.0)	0.2
G60	1144 (16.4)	1178 (9.0)	2.9	1175 (10)	2.7

Table 4.3. Viscosity results obtained from reference measurements and the proposed method. The standard deviation of the reference measurements are less than 1×10^{-4} mPa.s. Error is the percent difference between the reference and measured values

VISCOSITY	Reference	2 Cantilevers		3 Cantilevers	
Liquid	<i>Mean (Standard Deviation) [mPa.s]</i>	<i>Mean (Standard Deviation) [mPa.s]</i>	<i>Error (%)</i>	<i>Mean (Standard Deviation) [mPa.s]</i>	<i>Error (%)</i>
DI35	0.720	0.801 (0.001)	11.2	0.793 (0.001)	10.1
G5.2	1.062	1.031 (0.006)	2.9	1.029 (0.006)	3.1
E6	1.072	1.025 (0.010)	4.3	1.022 (0.010)	4.6
G13	1.336	1.347 (0.007)	0.8	1.350 (0.005)	1.0
G17	1.502	1.548 (0.007)	3.0	1.553 (0.009)	3.4
G25	1.911	1.934 (0.016)	1.2	1.940 (0.022)	1.5
G43	3.910	3.773 (0.242)	3.5	3.785 (0.234)	3.2
G60	9.990	5.660 (0.232)	43.3	5.670 (0.236)	43.2

4.4 Conclusion

A novel method to obtain viscosity and density separately with two (or more) cantilevers is developed. The frequency shifts due to viscosity and density changes are used in the calculation. The PLL-based frequency tracking enables fast and accurate detection which makes the system more practical compared to frequency sweep based methods using network analysers. The achieved accuracies for viscosity and density are well suited for blood and blood plasma measurements [95, 142]. Within the scope of this study we focused on proving the capability of the method to extract density and viscosity and did not force to achieve higher accuracy in a broader dynamic range. It is obvious that one can still employ our methodology to achieve higher accuracy and dynamic range by either choosing cantilevers which are optimized for the intended range or increasing the number of cantilever designs. Also the remote nature of actuation and sensing makes the system suitable for usage in single use and disposable cartridges, which is required for point-of-care applications.

5 COAGULATION TIME MEASUREMENTS

5.1 Introduction

Blood coagulation testing measures the time of onset of blood clotting, which involves an activating cascade of coagulation factors that ends with the formation of a cross-linked fibrin clot. Coagulation time tests are required for patients who are receiving anticoagulant therapy [144], undergoing pre-operation evaluation [145], or suffering from hepatic or renal disorders [146], monitoring the disease progression such as dengue hemorrhagic fever [147]. Also patients with a risk of embolism, stroke or atrial fibrillation require their coagulation time to be measured and adjusted periodically by suitable oral anticoagulants. Thus, fast, reliable and simple assays are needed to monitor the coagulation parameters. [16, 148]. Prothrombin time (PT) and activated partial thromboplastin time (aPTT) are the most frequently performed tests to determine the functionality of the blood coagulation system [149]. The measurement of PT and aPTT depends on activation by the tissue factor (extrinsic system) or surface (intrinsic system), respectively (White et al., 2003). In clinical use, aPTT is performed by manually mixing blood plasma samples with phospholipids and an activator (silica, celite, kaolin or ellagic acid). For the PT test, a reagent containing tissue factor is added to a plasma sample to induce coagulation through the extrinsic pathway.

In general practice, patients need to visit a hospital or a central laboratory periodically for coagulation tests. Such a procedure puts a significant burden on the health-care provider and increases the return time and the cost of the test. To alleviate these problems, some portable devices have been developed for PT measurements [18, 106]. Although these systems are practical and require low

sample volume, they cannot measure clot formation directly and they use a potentiometric measurement principle. Furthermore, these devices require electrical connections between the disposable cartridge and the analyzer unit which can create reliability problems in long-term use. On the other side, the system developed in this study measures the clot-time directly by monitoring the mechanical properties of blood with a non-contact actuation and detection method. Also it is the only platform that can perform multiple tests with the same sample.

Magnetoelastic sensors have been used to measure coagulation times [101]. Same group has also conducted PT tests by using the same principle [102]. However this kind of a system necessitates a Helmholtz coil with 33.2 cm diameter which causes a complexity to scale down the system for point of care use. The researchers also utilized Quartz Crystal Microbalance (QCM) to conduct PT tests and investigate the effect of different coagulation factors [10, 12, 150]. In these studies, frequency or dissipation of the QCM is tracked to detect coagulation times. The mixing of the blood samples with the reagents were conducted manually on the QCM sensors. Hence, functionalized sensors with microfluidic platforms are not proposed in these systems. Also as Harris et al. mentioned, due to the high cost of a quartz crystal resonator it is exorbitant as a single use disposable device. The re-use is also challenging as the adhesion of the biomaterials, such as fibrin, to the gold electrode surface of QCM. Xu et al. utilized Contour-Mode Film Bulk Acoustic Resonator (C-FBAR) based techniques for clot-time measurements [151]. Although they made successive clot-time measurements with varying calcium concentrations, the microfabrication complexity of C-FBAR devices are complex which increases the cost. An electrical impedance sensing based microfluidic device is also used for aPTT test [152]. Their method exploits the change in the magnitude and phase of electrical impedance of whole blood during coagulation for APTT measurement. The system requires electrical connection between the microfluidic channel and the lock-in amplifier which they used for

monitoring phase and amplitude. In all these MEMS based studies mixing of the blood samples with the reagents were conducted manually on the sensors. Hence, functionalized sensors with microfluidic platforms are not proposed.

A commercial device [153] based on static mode microcantilevers is in preparation for launch soon. These cantilevers require electrical connections to the cartridge due to the use of piezo-resistive elements for sensing and actuation [154]. Microfluidic devices is also used for aPTT test [152] and PT test [107]. To the best of author's knowledge, the studies in the published literature are limited to single tests and not multiplexed. Most of the clinical applications require, both PT and aPTT tests. A MEMS based microfluidic device enabling both tests with one sample is a promising tool for Point-of-Care.

In Chapter 2 and Chapter 3, blood plasma viscosity measurements through phase measurements with high sensitivity using the proposed scheme [141, 142] were conducted. During blood coagulation, a sudden change in the blood plasma viscosity occurs due to fibrin generation [10]. By monitoring the viscosity over a course of time, sudden increase in viscosity can be used as an indication of coagulation time for the given sample.

In this chapter a novel method using the microcantilevers and the sensing method for fast and convenient blood coagulation time measurements is introduced. To author's knowledge, this is the first study using dynamic-mode cantilevers for coagulation time monitoring. The principle of the assay was introduced and the assay was tested with known plasma controls and the results are compared with reference datasheet and reference commercial device. Additionally, for further implementation, the microfluidic channels of cartridge are functionalized for PT and aPTT tests by drying specific reagents in each channel. PT and aPTT testing of

control plasma samples revealed that coagulation times could be successfully detected for normal and different abnormal plasma controls.

5.2 Materials and Methods

5.2.1 The modification of the chip designs and microfabrication

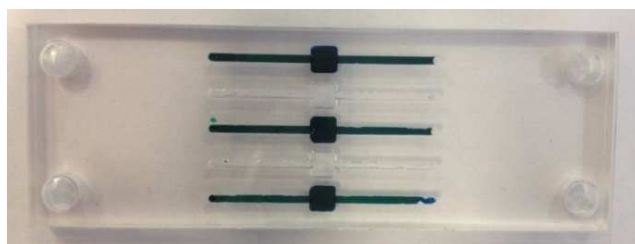
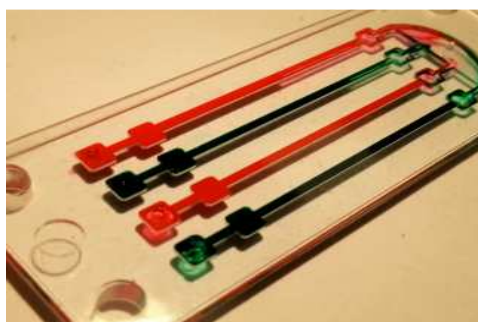
Sensor fabrication was performed using a procedure as reported in section 2.2. The fabricated nickel cantilevers are without grating and have 200 μm length, 20 μm width and 2 μm thickness. These dimensions are chosen to keep the resonant frequency within the operating frequency of the lock-in amplifier that is used in the experiments. The main modification is on chip geometry. In order to obtain multiplexed measurements in a small cartridge area the chip dimensions are reduced from 6 mm x 8 mm to 2.5 mm x 2.5 mm. The fabricated nickel electroplated mask and the final dies are shown in Figure 5.1.



Figure 5.1: a) The wafer containing 2.5 mm x 2.5 mm chips b) the diced dies

After the microfabrication, the MEMS dies are placed into microfluidic cartridge. The microfluidic cartridge consists of two parts: the substrate and the lid. Both of the parts are made of poly methyl methacrylate (PMMA) and the specific geometries on

each are patterned by precision machining. MEMS dies were bonded into the wells on the substrate. UV cured epoxy (Norland Optical Adhesive 81) was used for bonding. The microfluidic channels are patterned on the lid part. Each microchannel has 200 μm depth and 1 mm width. These channels can be functionalized for different tests. The lid and substrate are bonded to each other by UV cured epoxy. This type of epoxy bonding also provides excellent sealing between the parallel channels. The sealing of the cartridges are tested with colored ink as shown in Figure 5.2a. The one shown in Figure 5.2a is an earlier cartridge design to test the sealing efficiency whereas the one in Figure 5.2b is the final cartridge design for multiplexed coagulation time measurements. In Figure 5.2c another cartridge design with one inlet is also shown. Each channel has a total volume in of 10 μl (Figure 5.2b). This volume can be further reduced with optimized cartridge geometry.



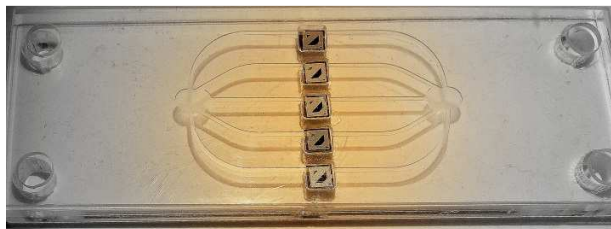


Figure 5.2. The cartridges for multiplexed coagulation measurements. a) An earlier design. b) the tested design. c) the design with single inlet.

5.2.2 The measurement principle

The schematic of the experimental setup and the principle of operation are shown in Figure 5.3. The PMMA cartridge contained the MEMS dies with a $2.5 \text{ mm} \times 2.5 \text{ mm}$ size. The nickel cantilevers were actuated magnetically with a coil placed in close proximity to the backside of the PMMA flow cell. Since no direct electrical contacts are required on the chip, magnetic actuation with an external coil has a significant advantage for in-liquid operation. The optical read-out can also be conducted remotely with laser based techniques as shown in 2.3.2. The results presented in this study were obtained with a Laser Doppler Vibrometer (LDV). The phase difference between the coil drive and the LDV was monitored with a lock-in amplifier (Zurich Instruments HF2LI) and recorded simultaneously using a LabVIEW interface. All experiments were conducted at $37 \pm 0.1 \text{ }^{\circ}\text{C}$. The cartridge with parallel channels and the temperature controller unit is shown in Figure 5.3. The temperature unit is same with the one shown in (Figure 2.20)

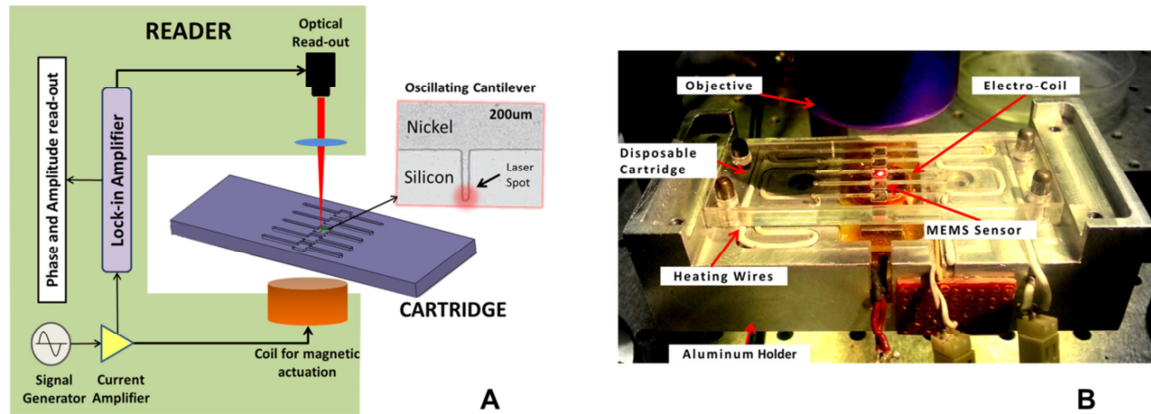


Figure 5.3 a) Schematic view of the measurement setup. b) Photo of the setup.

5.2.3 PT and aPTT measurements with control plasma samples

PT and aPTT measurements were performed on a healthy donor's plasma samples and commercially available control plasma samples.

For PT and aPTT Tests, Calcium Freeze Dried Rabbit Brain Thromboplastin (DIAGEN, UK, Lot No: T74) and Micronised Silica Platelet Solution (Lot No: SP63, DIAGEN, UK) reagents are used, respectively. Three different control plasma samples used were The Normal (Lot No: KP93, DIAGEN, UK,), Abnormal 1 (Lot No: AB1-80, DIAGEN, UK,) and Abnormal 2 (Lot No: AB2-79, DIAGEN, UK,). The blood samples were taken from a healthy 28 year old male donor into a citrated vacutainer tube (Greiner Bio One Vacuette 454326 Polyethylene Therephthalate Sodium Citrate Blood Collection Coagulation Tube, 3.2% Sodium Citrate). Afterwards the plasma is separated with centrifugation at 5000 g x 10 min. Before measurements, all the reagents and samples were pre-warmed at 37 °C.

5.2.4 Sample preparation for scanning electron microscopy (SEM)

Samples were prepared as described for the test on the MEMS chip and then fixed for 4 h in 2.5% glutaraldehyde at room temperature. Dehydration with increasing ethanol concentrations (30%-100 % v/v) was followed by drying. The samples were coated with 3 nm Au-Pd and imaged by Zeiss Ultra Plus FE-SEM.

5.2.5 Characterization of the system for coagulation measurements

When the cantilevers are operated in a viscous liquid media, the viscosity influences the resonant frequency and the quality factor of the system. In Chapter 2 and Chapter 3 the viscosity changes with a sensitivity of 0.01 mPa.s were measured, by tracking frequency at constant phase difference or by tracking phase changes at constant frequency. During blood coagulation a sudden change in blood plasma viscosity occurs. When the coagulation is initialized, thrombin acts on fibrinogens that result in the fibrin generation, then fibrins aggregate and form insoluble clots. After coagulation measurement, MEMS chips were investigated with SEM and clot formation was clearly observed on MEMS cantilevers (Figure 5.4). Changes in the viscosity of plasma were caused by the formation of the fibrin clot [150] and this significant change in the viscosity was monitored by real-time tracking of the phase and amplitude.

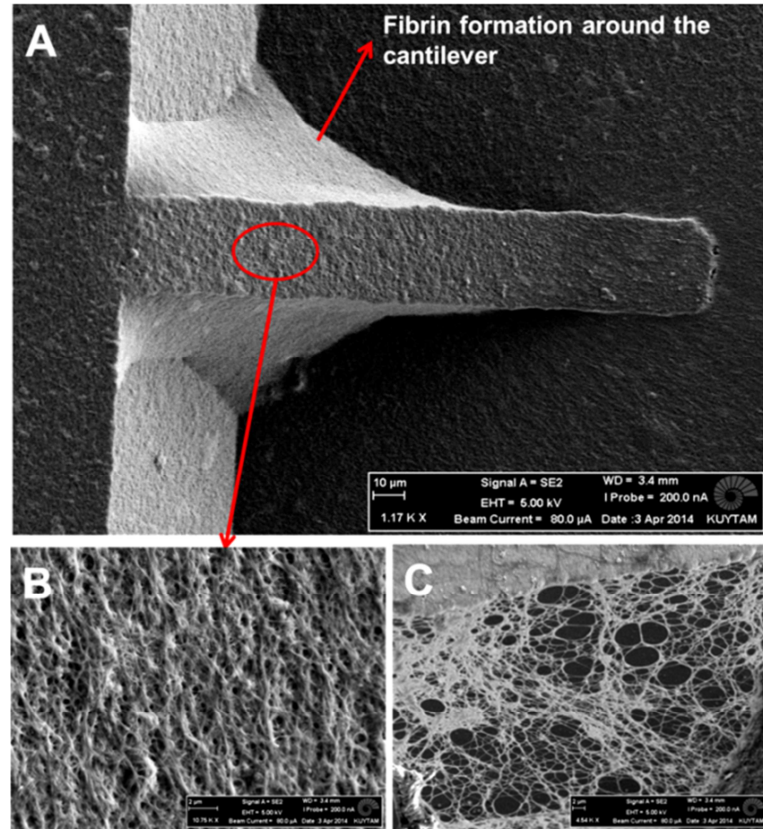


Figure 5.4: a) SEM images of fibrin networks formed after the coagulation on the MEMS chip b) more detailed c) less concentrated form of the fiber structure.

Before the coagulation measurements the system was characterized in air, Deionized (DI) water and blood plasma. Figure 5.5a shows the frequency response and the phase of the system in different mediums. The resonant frequency of the cantilever is 39206 Hz when it is operated in air. Also the Quality Factor (Q) is around 178. However when it was operated in DI water the frequency drops down to 22185 Hz and the Q is around 5. This harsh decrease is due to hydrodynamic loading which has both inertial and viscous components [36]. The response in blood plasma before coagulation is similar to DI water. The resonant frequency is 20267

Hz and the Q is around 3.6. This is due to the higher viscosity and density of blood plasma with respect to DI water as shown in section 2.1.2.

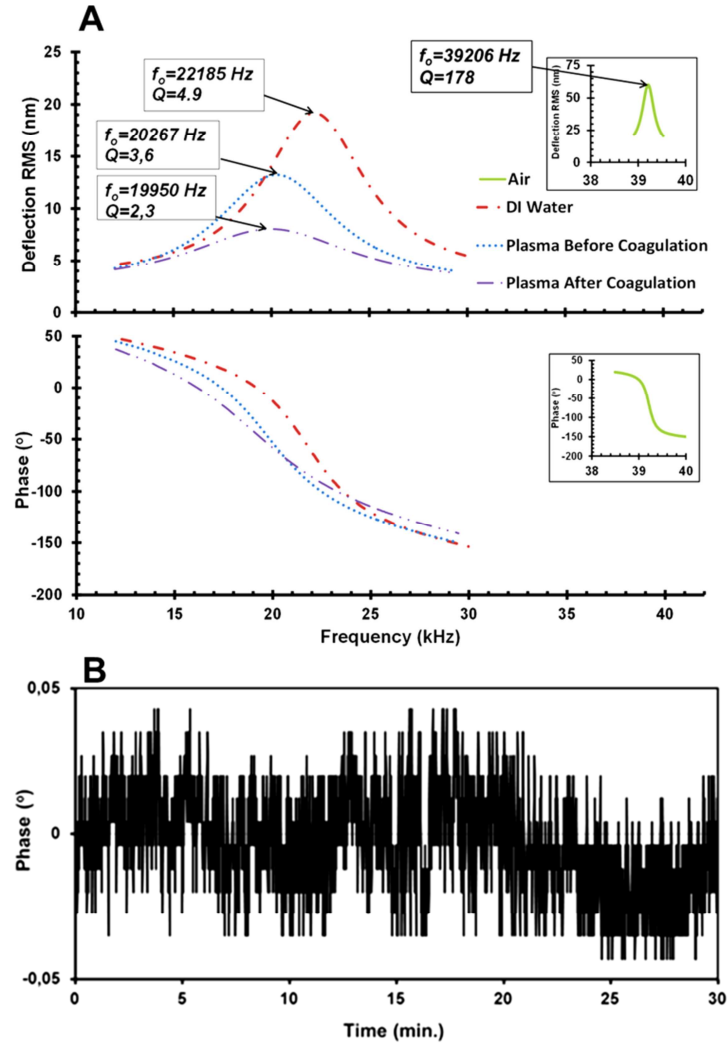


Figure 5.5: a) Frequency response and phase of the system in air, in DI water and in human blood plasma before and after coagulation. b) Phase stability results for citrated (non-coagulating) blood plasma $f = 20$ kHz, standard deviation = 0.015° .

During blood coagulation, a sudden change in the blood plasma viscosity occurs due to fibrin generation [10]. This kind of viscosity change creates an increase in the hydrodynamic loading of the vibrating structures in viscous liquids according to Sader's theory [36]. For example, increasing viscosity reduces the vibration amplitude of the vibrating mechanical structure driven by constant amplitude. Also, the effect of viscosity change can be observed as a phase shift between the drive signal and the phase of the mechanical displacement. Depending on the drive frequency the phase shift could be either positive or negative. The effects of viscous loading on mechanical resonators have been further investigated in the literature [50, 113, 155]. After coagulation, the quality factor and the resonant frequency is changed. The significant difference in the phase curve after the coagulation is shown in Figure 5.5. According to the proposed method the system was excited at a certain frequency around the resonant peak. The phase difference at that certain frequency changed during coagulation. Hence simultaneous monitoring of the phase gave a unique signature which indicates the coagulation time.

Figure 5.5b shows the long-term phase stability of the system in citrated blood plasma. The cantilever was driven at its resonant frequency. The phase was recorded for 30 minutes. The standard deviation of the phase during this measurement is 0.015° which can be defined as the noise floor of the system. As will be shown during coagulation measurements, this value is highly sufficient for sensing blood coagulation.

5.3 Measurements with Control Plasma Samples

Both the aPTT and PT tests are conducted with the proposed system. In order to investigate the sensitivity and reproducibility of the system, commercially available control plasma samples were used. The coagulation time measurements with

Normal, Abnormal 1 and Abnormal 2 control plasma samples are conducted and the results are compared with the standard values provided by the manufacturer and a commercial reference device CoaguCheck XS (Roche, Germany). Afterwards different channels of the same cartridge are functionalized for different tests and both PT and aPTT tests are performed on the same cartridge.

5.3.1 aPTT Test Protocol

- **STEP 1:** Set the temperature of the setup to 37°C and wait 5 minutes to settle
- **STEP 2:** Heat the plasma samples, Micronised Silica/Platelet Substitute Mixture reagent and 25 mM CaCl₂ to 37°C in an external heating bath
- **STEP 3:** Mix the 10 µl of reagent and the 10 µl of plasma sample in an Eppendorf tube and wait for 5 minutes for incubation. Ensure the perfect mixing with gently pipetting.
- **STEP 4:** Mix the solution with 10 µl 25 mM CaCl₂. Ensure the perfect mixing with gently pipetting. Immediately start the clock (t=0)
- **STEP 5:** Immediately place the 5 µl of the mixture on the MEMS chip.
- **STEP 6:** Start recording the phase difference between the coil input and sensor output. Note the value on the clock. (The coil is driven at a frequency which was defined during characterization. The frequency doesn't need to be the exact peak frequency since the phase change during coagulation is a huge signal and the time at the onset of the coagulation can be monitored even though the system is not working at exact resonant frequency. Also the focus of the laser should be adjusted prior to experiment)
- **STEP 7:** Monitor the sharp phase change and record data for 10 more seconds after stabilization.

- **STEP 8:** Report the quantitative value of the onset of the coagulation using the developed MATLAB routine as shown in Appendix. (Do not forget to add the time delay during placing)
- **STEP 9:** Remove the MEMS chip from the cartridge and throw it to the medical waste bin

5.3.2 PT Test Protocol

- **STEP 1:** Set the temperature of the setup to 37°C and wait 5 minutes to settle
- **STEP 2:** Heat the plasma samples, Calcium Freeze Dried Rabbit Brain Thromboplastin reagent to 37°C in an external heating bath
- **STEP 3:** Mix the 20 μ l of reagent and the 10 μ l of plasma sample in an Eppendorf tube, ensure the perfect mixing with gently pipetting.
- **STEP 4:** Immediately start the clock ($t=0$)
- **STEP 5:** Immediately place the 5 μ l of the mixture on the MEMS chip. (the operator should move fast since the PT time is very low)
- **STEP 6:** Start recording the phase difference between the coil input and sensor output. Note the value on the clock. (The coil is driven at a frequency which was defined during characterization. The frequency doesn't need to be the exact peak frequency since the phase change during coagulation is a huge signal and the time at the onset of the coagulation can be monitored even though the system is not working at exact resonant frequency. Also the focus of the laser should be adjusted prior to experiment)
- **STEP 7:** Monitor the sharp phase change and record data for 10 more seconds after stabilization.

- **STEP 8:** Report the quantitative value of the onset of the coagulation using the developed MATLAB routine as shown in Appendix. (Do not forget to add the time delay during placing)
- **STEP 9:** Remove the MEMS chip from the cartridge and throw it to the medical waste bin

5.3.3 Results

The phase and amplitude of the system during a PT test is shown in Figure 5.6a. The sharp change of the phase and amplitude during coagulation can be seen. The first 5 seconds is the time passed during the mixing of reagent and plasma and introducing the mixture to the system. The amplitude data is noisier than the phase read-out. This can be related with the relatively small vibration amplitudes. The RMS value of the displacement is in the order of 10 nm. This is a very small value compared to cantilever dimensions (Length: 200 μ m, Width: 20 μ m, Thickness: 2 μ m). Also during coagulation, as a result of increased viscosity, the vibration is being damped and the amplitude decreases to even lower values. The amplitude read-out is susceptible to the environmental noise. However the phase read-out approach is more immune to external noise sources since a reference signal (coil input) is being used. Phase gives clearer information since the Signal-to-Noise Ratio (SNR) is higher. It is more convenient to use phase for tracking coagulation.

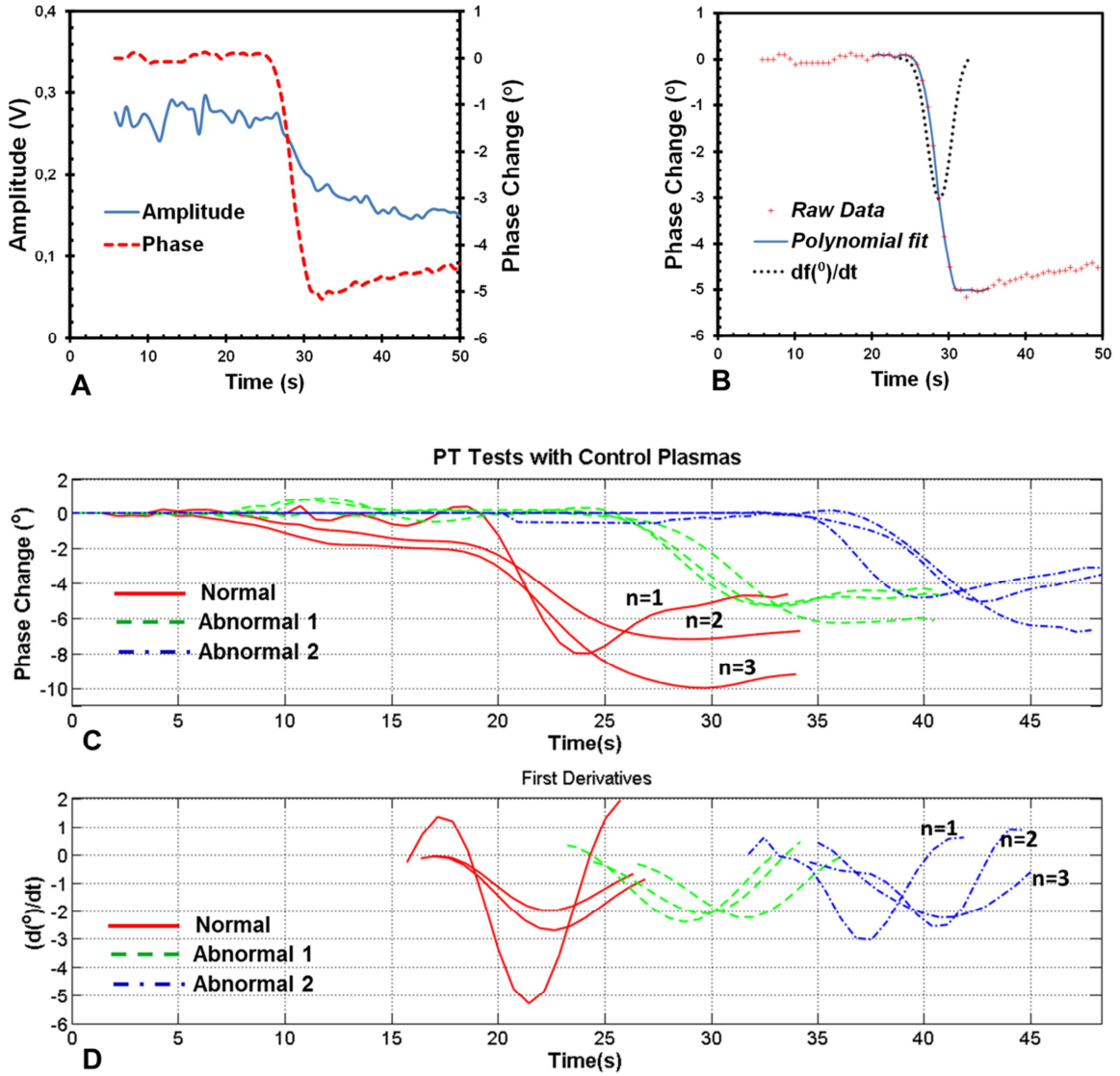


Figure 5.6: a) The change of the phase and amplitude during PT test with an Abnormal 1 control plasma sample. b) Polynomial fit to the phase data and the first derivative of the fit function (Appendix). c) PT tests with normal, abnormal 1 and abnormal 2 control plasmas $n=3$ for each measurement. d) First derivatives of the phase curves.

After the data are collected a polynomial is fitted to the phase data which is shown in Figure 5.6b. When the coagulation starts the dramatic change in the phase data also begins. In order to obtain the exact start point the first derivative of this polynomial is used to obtain quantitative data. The time where the derivative is zero ($df/dt \rightarrow 0$) indicates the coagulation start time whereas the local minimum ($df/dt \rightarrow \min.$) shows the mid-point of the coagulation process (Figure 5.6b). The start point of the sharp decrease is local extremum. In Figure 5.6c the fit functions obtained from the repetitive PT measurements with Normal, Abnormal1 and Abnormal2 plasma are shown. Figure 5.6d shows the first derivatives of these functions. The difference between the coagulation times with different samples can be seen. They can be clearly distinguished from each other. The same data acquisition and processing protocol is also applied in aPTT measurements. After the measurements, the values are normalized with respect to the mean value of the normal plasma sample. The commercial devices report the coagulation times in terms of International Normalized Ratio (INR) [16]. There will be a variation between the test results (in seconds) of a normal individual. This is due to the variations in the batches of reagent and test conditions. The INR is devised to standardize these results. It is related between the tested individual's PT time and normal PT time [107]. International Normalized Ratio (INR) for PT measurements and normalized ratio for the aPTT measurements are obtained. The mean value, standard deviation and coefficient of variation for the PT and aPTT measurements are shown in Table 5.1.

The start-point of the coagulation obtained with the system has a better coherence with the reference data than the coagulation mid-point. Also another reference PT measurement is conducted with a reference commercial device CoaguCheck XS. Again the coagulation times are in a good agreement with the commercial reference

device measurements. The proposed novel assay can significantly differentiate coagulation times between normal, abnormal 1, and abnormal 2 samples ($p < 0.01$).

A reliable diagnostic assay should yield the same measurements for the same target. Various sources of variability affect overall cartridge variability: electronic and fabrication chip-to-chip variability, and biological assay variability. All three of these variability sources contributed to the overall measurement variability. To check the reproducibility of the assay, all the measurements were repeated three times in a day and in three different days. The percent coefficient of variation (CV %) for the tested samples are 7.28 and 6.33 for PT and aPTT measurements, respectively. These reproducibility values are comparable with the commercial devices available in the market [156] and recent studies in the literature [107]. The results for aPTT and PT measurements in comparison with the reference value are shown in Figure 5.7. There is no statistical difference between the results from the proposed system and those of reference device and control samples data sheet ($p > 0.05$).

Table 5.1: PT results in terms of INR and aPTT results in terms of normalized ratio.

PT (INR)									
	MEMS results (INR)						Datasheet (INR)	Reference Device (INR)	
	Within Day			Between Day					
	Mean	σ	CV (%)	Mean	σ	CV (%)	Mean	σ	
Normal	0.95	0.07	7.73	1.00	0.08	7.79	1.03	0.07	1.30
Abnormal 1	1.47	0.16	10.54	1.60	0.11	6.85	1.74	0.35	1.60
Abnormal 2	2.74	0.26	9.63	2.75	0.20	7.20	2.83	0.40	3.30
aPTT (Normalized Ratio)									
	MEMS results (Ratio)						Datasheet (Ratio)		

	Within Day			Between Day			Mean	σ
	Mean	σ	CV (%)	Mean	σ	CV (%)		
Normal	1.04	0.05	5.24	1.01	0.04	3.94	1.01	0.08
Abnormal 1	1.19	0.04	3.10	1.19	0.11	9.11	1.36	0.20
Abnormal 2	1.89	0.20	10.65	1.78	0.10	5.87	1.93	0.28

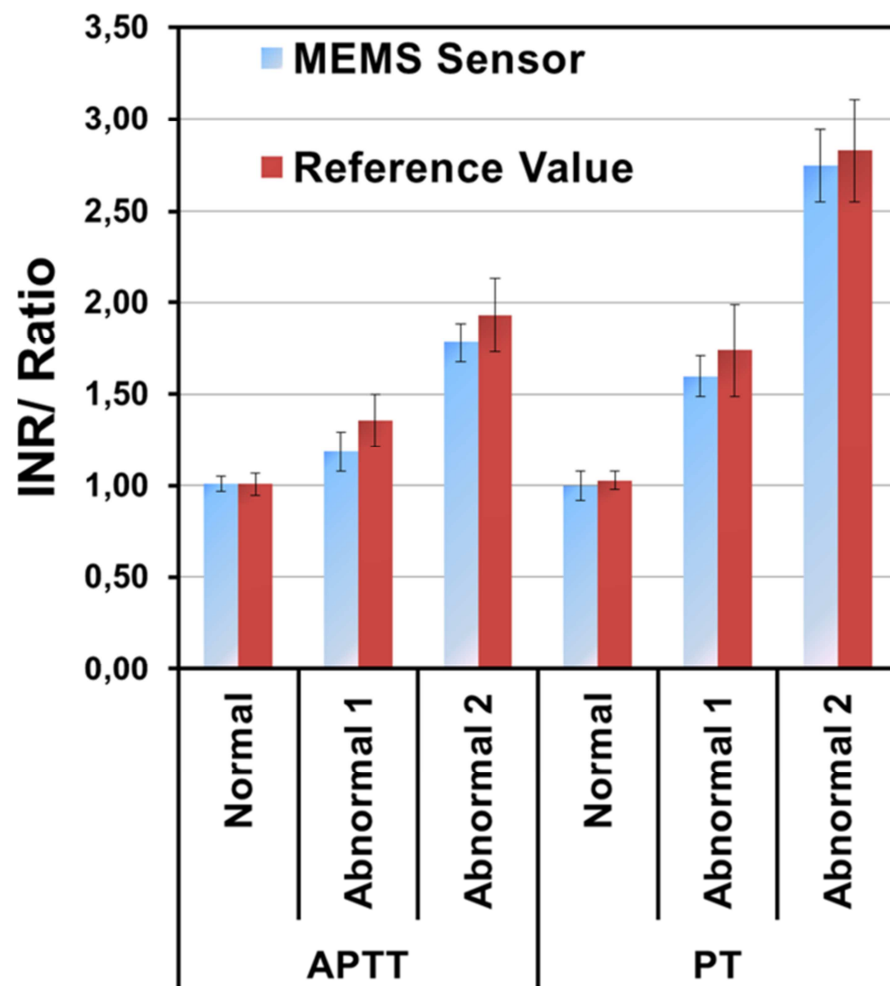


Figure 5.7: The INR and Normalized Ratio values of PT and aPTT tests. The reference value is provided from the manufacturer's data sheet.

5.4 Measurements with surface functionalized channels

All above measurements were performed with samples prepared outside the cartridge and placed on MEMS chips. For further implementation, the microfluidic channels of cartridge are functionalized for PT and aPTT tests by drying specific reagents in each channel. Then PT and aPTT measurements were performed with surface functionalized cartridges. The surface functionalized channels of the cartridge are shown in Figure 5.8a. A photo of a surface functionalized cartridge with parallel channels is shown in Figure 5.8b. For the aPTT Test, Micronised Silica Platelet Solution (Lot No: SP63, DIAGEN, UK) was used whereas for the PT Test Freeze Dried Rabbit Brain Thromboplastin (Lot No: DT139, DIAGEN, UK) was used.

5.4.1 Functionalization Protocol

- **STEP 1:** Pour 10 μ l of reagent to the corresponding channel.
- **STEP 2:** Pour 10 25 mM CaCl_2 of reagent to the opposite channels (Figure 5.8a).
- **STEP 3:** Place the cartridge in a vacuum chamber or desiccator for drying
- **STEP 4:** Keep the cartridge at 100mmHg vacuum for 20 min and observe the drying (this is a safe value, for lower pressures the time required will decrease. Warning: The very low vacuum values may harm the PMMA cartridge; this should also be taken into account).
- **STEP 5:** Break the vacuum and take out the cartridge.
- **STEP 6:** Bond the PMMA lid to the cartridge with epoxy, gently.
- **STEP 7:** The functionalized cartridge is ready to use. Either plug the cartridge to the heater unit and conduct the test as soon as possible or keep it in a non-humid environment.

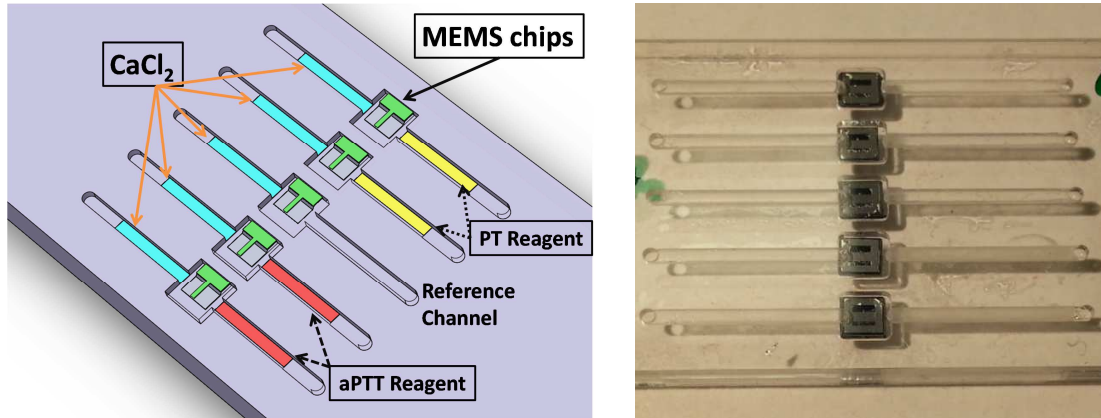


Figure 5.8: a) The scheme of the functionalization procedure. b) Photo of the surface functionalized channels.

For each test 10 μl of blood plasma sample at 37°C is poured into the reagent coated channel. For aPTT test 5 min of incubation is chosen whereas for the PT test this time is 1 min. After the incubation, the sample is pushed to the opposite channel that is coated with CaCl₂. When the sample touches to the channel walls, the sample is pulled back to the cantilever site and the clock is started ($t=0$). Real-time phase and amplitude read-out was conducted. Though the tests are conducted one at a time within the scope of this paper, tests can be performed simultaneously using multiple photodetectors for the readout. Since the electromagnetic actuation is at a constant frequency, using a detector array will enable simultaneous monitoring of the phases of different cantilevers in parallel channels.

5.4.2 Test Protocol

- **STEP 1:** Set the temperature of the setup to 37°C and wait 5 minutes to settle.
- **STEP 2:** Heat the plasma samples, to 37°C in an external heating bath.
- **STEP 3:** Pour 10 μl of blood plasma sample to each channel.

- **STEP 4:** Wait 5 minutes for incubation for the aPTT test, 1 min for incubation for PT test.
- **STEP 5:** Push the incubated sample with gentle pipetting to the opposite channel that is coated with CaCl_2 .
- **STEP 6:** When the sample touches to the walls of the CaCl_2 channel pull the sample back to the cantilever site and immediately start the clock. ($t=0$)
- **STEP 7:** Start recording the phase difference between the coil input and sensor output. Note the value on the clock. (The coil is driven at a frequency which was defined during characterization. The frequency doesn't need to be the exact peak frequency since the phase change during coagulation is a huge signal and the time at the onset of the coagulation can be monitored even though the system is not working at exact resonant frequency. Also the focus of the laser should be adjusted prior to experiment).
- **STEP 8:** Monitor the sharp phase change and record data for 10 more seconds after stabilization.
- **STEP 8:** Report the quantitative value of the onset of the coagulation using the developed MATLAB routine. (Do not forget to add the time delay during placing).
- **STEP 9:** Remove the cartridge and throw it to the medical waste bin.

5.4.3 Results

The results of the PT and aPTT test proceeded on the same cartridge is shown in Figure 5.9. As expected, when the coagulation started a sharp change in the phase was observed. The reference curve is obtained by addition of Ca^{2+} to the citrated blood plasma. According to the first derivative of the phase data the coagulation time is 645s for the reference channel. This is an expected value since no external

reagent is added to the system. According to the first derivative of the phase data the coagulation time for aPTT test was 67s whereas for PT test it was 22s. According to the reference values in the reagent datasheets these values are supposed to be between 35-40s and 14-17s for aPTT and PT tests, respectively. This slight difference was probably due to reagent mixing inefficiency in microchannels and further improvements are needed to obtain more accurate PT and aPTT values.

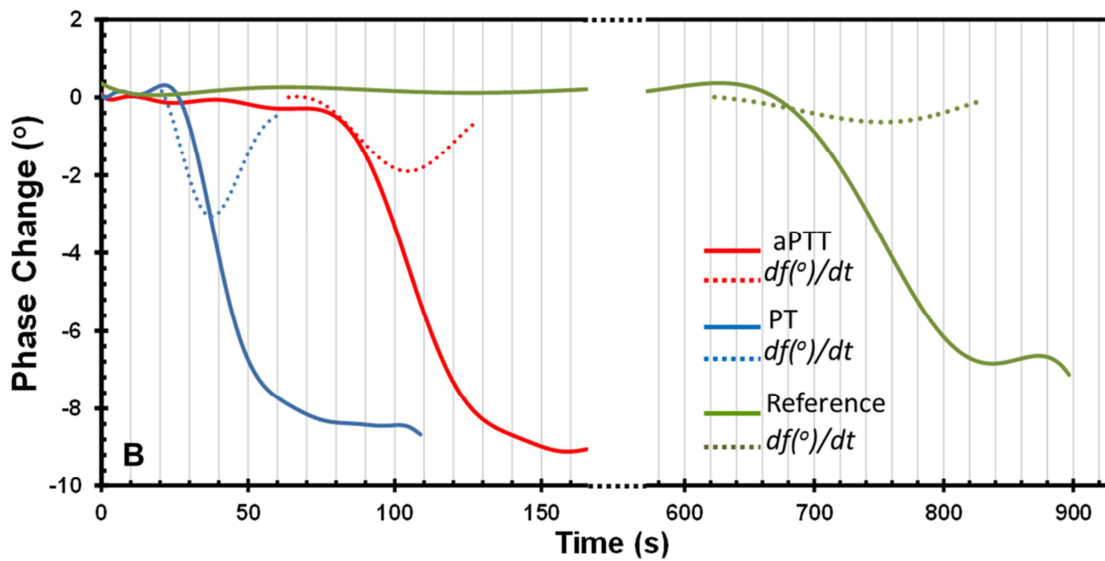


Figure 5.9: aPTT and PT tests and the reference measurement conducted in different channels of the same cartridge.

5.5 Conclusions

In this chapter the use of the system as a sensor array platform enabling multiple coagulation tests is demonstrated. In different channels of the cartridge aPTT and PT tests were conducted with blood plasma taken from a healthy donor. Protocols for different coagulation tests are developed. Commercially available reagents were used. In both tests, fibrin generation and end of coagulation process were identified

clearly. Both the phase and the amplitude can be used for monitoring the coagulation; however phase measurements are more immune to noise and give a clearer signal. The sample volume used for each test was 10 μ l which could be decreased with further design improvements. In order to improve the system to commercial medical device level, standardized clinical tests are required. Nevertheless, obtained results gave satisfactory information about the proof of concept. This is the first demonstration of the use of a dynamic microcantilever based system for coagulation time measurement. The independence of the cartridge and reader unit increases the potential of this approach as a Point-of-Care system.

6 PROTEIN AND MASS DETECTION

6.1 Introduction

In this chapter, the mass detection capability of the system was tested. The LDV setup, the cartridge and MEMS chip construction was modified for multichannel experiments. Microcantilevers in different channels of one cartridge were functionalized with 4 different antibodies, Hepatitis B, Hepatitis C, HIV and Syphilis. Afterwards the antigens of these viruses are introduced to the channels and detected. The concentrations of the antigens were 1 mg/ml. Prior to these experiments the system is tested with microbead detection experiments using biotin streptavidin bonding. The measurement principle is again based on phase tracking at a constant frequency. The phase is tracked with the Lock-in amplifier. When the binding occurs it creates a shift in resonant frequency and phase difference. A reference channel is utilized to avoid the background fluctuations in the phase stability. In the first part the modifications in sensor and fabrication is shown. Afterwards the functionalization process is described. Finally the results of the biological experiments are presented.

6.2 Materials and Methods

6.2.1 The modification of the chip and cartridge designs and microfabrication

Sensor fabrication was slightly modified in this chapter. In order to conduct functionalization for biological agent binding the surface should be gold. Hence an additional gold coating on top of the nickel layer was required. For this purpose two new steps are added as shown in Figure 2.4. The first new step is lithography with a second mask and the second step is the lift-off. After nickel electroplating, gold etching is applied. Then 6 μm thick positive photoresist, AZ9260 is coated with

spin-coating at 1000 RPM for 60 seconds with SPS Spin150 manual spin-coater. After coating a 2 minute post bake is applied at 100°C. After baking a second mask in which only cantilever geometries are transparent is aligned to the wafer using Karl-Süss MA45 mask aligner. The cantilevers have 200 µm length, 20 µm width whereas the mask openings corresponding to cantilevers were 200 µm long and 35 µm wide. This margin is necessary to obtain a good covering for all the cantilevers on the wafer and avoid misalignment. After alignment the wafer is subjected to UV light with intensity of 22 mW/cm². The exposure time is determined as 60 s with contact printing option. Afterwards the development was conducted with AZ351B Developer (Microchem. batch: DEAZ056342) in 1 min. Afterwards a 50 nm thick gold layer is sputtered on the wafer using RF sputtering with Vaksis PVD Handy sputter tool. Afterwards the lift-off process is conducted. Ultrasonication is applied for 30 seconds to initialize the process and the wafer is kept inside acetone for 5 hours. After the lift-off, the wet etching of Cr and anisotropic wet etching of Si is conducted and the cantilevers are released. The gold coated gold surfaces are shown in Figure 6.2. The residues of the alignment margins are also visible.

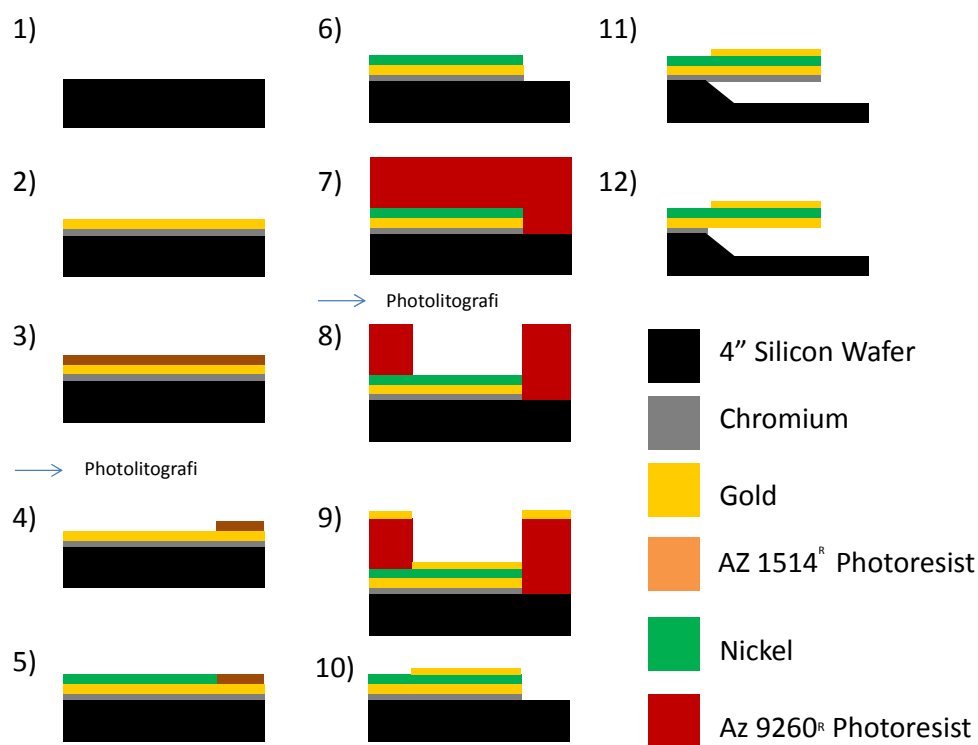


Figure 6.1: The modified fabrication scheme for top gold layer coating

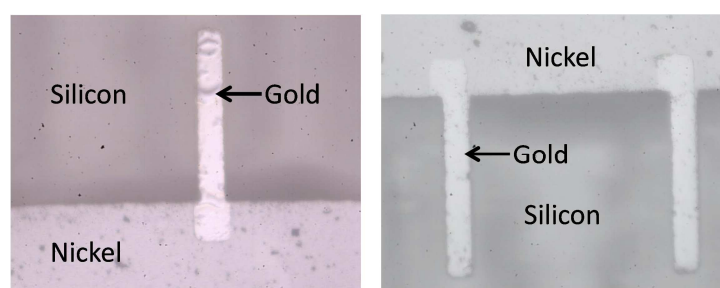


Figure 6.2: The gold coated cantilevers. Some gold layer is overflowed to the anchor due to alignment margins.

In this chapter, the MEMS dies and the cartridges were also modified for parallel read-out. A new mask is designed as shown in Figure 4.2a. Different from the previous designs the anchor surface is smaller as shown in the zoomed view in Figure 4.2b. The cantilevers have 20 μm width, 200 μm length and 2 μm nickel thickness.

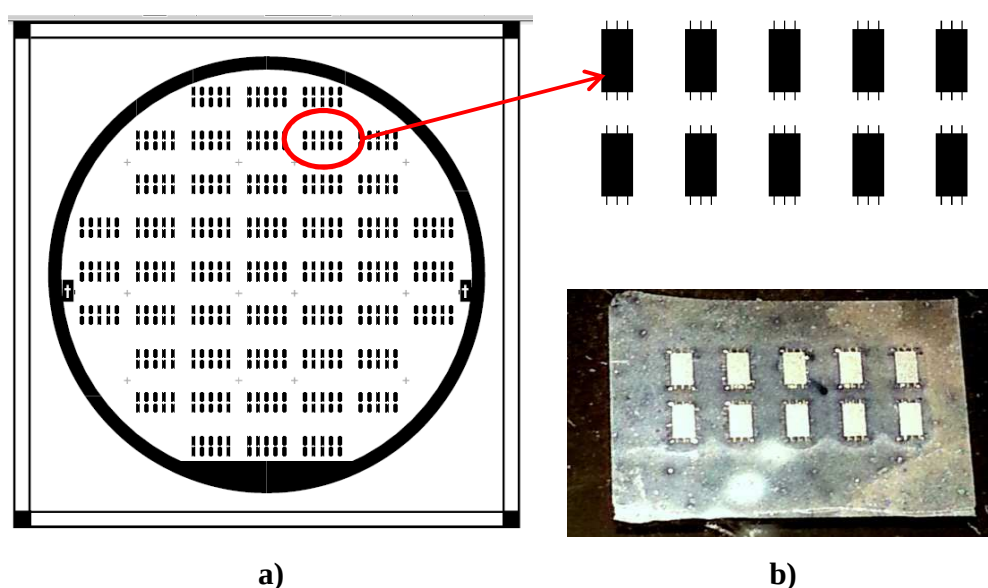


Figure 6.3. a) The new mask design for parallel readout. b) Zoomed view of one mems die and fabricated mems die.

The cartridge design for parallel read-out which was presented in Chapter 4 had some limitations over biological functionalization and optical read-out. First of all during functionalization some amount of antibody was non-specifically binding to the PMMA side walls of the channel and during the tests this time a certain amount of antigen was binding to these antibodies. This created a certain loss of biological agents in the solution and inhibited the detection. In the new cartridges a

biocompatible tape (Adhesives Research) is facilitated to form the channel geometries. The layers and the construction of the cartridge is shown in Figure 6.4a and b. The second problem was about the alignment of the cantilevers in parallel channels. In the previous design the MEMS dies were placed manually to the parallel channels hence it was a tedious work to align them in a perfect way and sometimes even not possible. The new design proposes alignment at the lithography stage to overcome this difficulty. The gap between each cantilever in parallel channels is constant (2 mm) and they are inherently aligned. There are 12 cantilevers in each row to provide higher number of good condition cantilevers in each channel. Also two or three MEMS dies are used together in one cartridge for ease of handling. The previous cartridge design and the modified cartridge design are shown in Figure 6.4c and d, respectively.

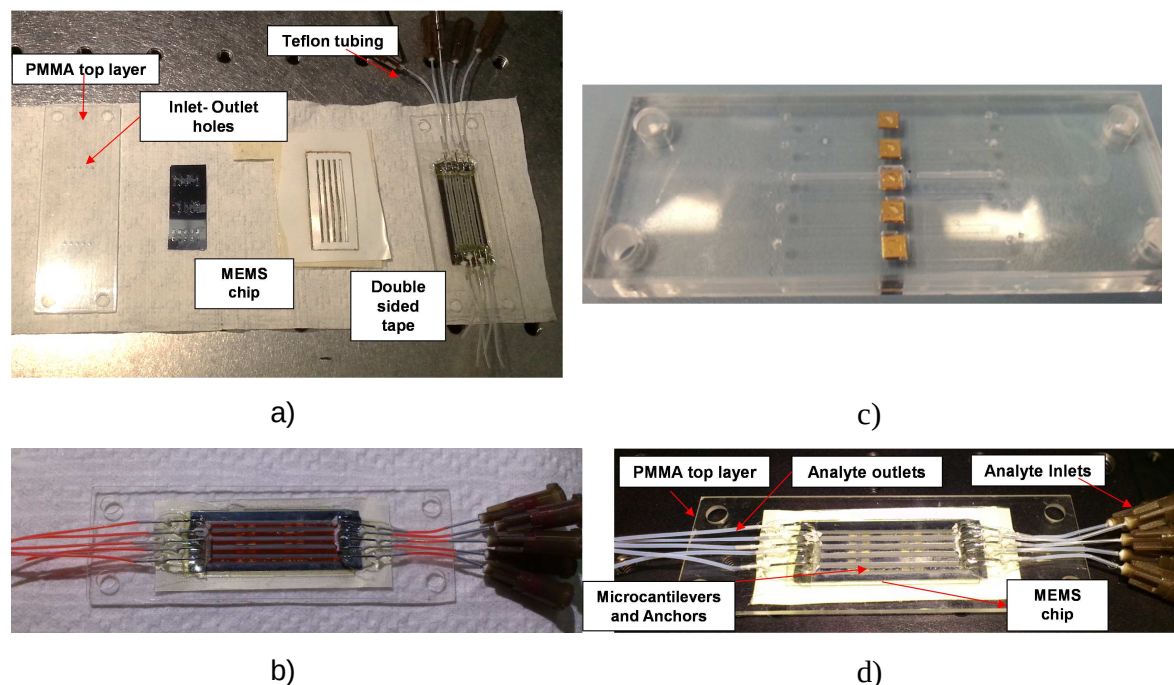


Figure 6.4. a) The construction and components of the cartridge. b) The sealing check of the cartridge with colored liquids. c) The previous cartridge design with standalone cantilevers in each channel which requires a tedious manual alignment. d) The cartridge structure.

6.2.2 The measurement setup

The LDV and lock-in amplifier based measurement principle described in Chapter 2 is used with small improvements to enable quasi simultaneous read-out from the parallel channels. The results are obtained with a Laser Doppler Vibrometer (LDV). The phase difference between the coil drive and the LDV was monitored with a lock-in amplifier (Zurich Instruments HF2LI) and recorded simultaneously using a LabVIEW interface. All experiments were conducted at 37 ± 0.1 °C. In order to scan the cantilevers during experiments a new experimental setup is constructed. In this setup the cartridge and the aluminum block in which the

cartridge is placed is fixed to the piezoelectric stages (PI nano stage) which enables motion in X and Y axis with a precision of 100 nm. The LDV is fixed and the cartridge is moving. To control these stages a custom made LabView code is utilized. Prior to the tests the cantilever coordinates are defined to the program, and during the test the stage scans these coordinates. At each cantilever the laser spot stays for 6 seconds and records 13 data points. After taking data from the first channel for 6 seconds, the stage is moved and the laser of the LDV is illuminated to the cantilever in the next channel. After all of the cantilevers in five channels are measured the stage turned back to its initial position.

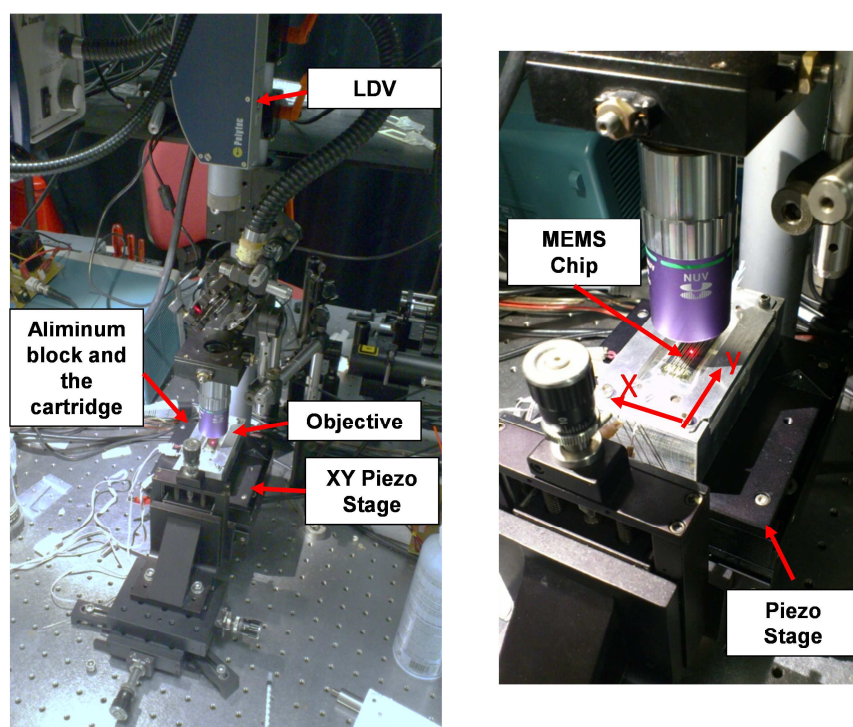


Figure 6.5 Photo of the measurement setup. The cantilevers are scanned using the piezo stage. Piezo stage control and the data monitoring are conducted with Labview interface.

6.2.3 The preparation and functionalization of the cantilever surface for agent detection

6.2.3.1 Antibody antigen experiments

At first the gold cantilever surfaces are coated with DSP (Dithiobis (Succinimidyl Propionate)) cross-linker polymer. This enables the binding of antibodies to the gold surface [43, 44]. For this purpose 0.004 g DSP is solved inside 1 ml of DMSO (Dimethyl Sulfoxide) and a DSP solution with 1mg/ml concentration was obtained. The MEMS surface were cleaned with oxygen plasma for and then dipped into the DSP solution for overnight and incubated. In order to remove the unbounded DSP particles it is rinsed 3 times with DMSO and PBS. This procedure is conducted for every channel on the cartridge, except for the reference channels if existing. Afterwards the antibodies are introduced to the microchannels and incubated for 3 hours. After PBS rinsing and measuring the phase difference between the coil and the LDV readout in the PBS, the related antigens were introduced to the corresponding channels. The phase shifts are measured. The antigen and antibody pairs tested are shown in Table 6.1

Table 6.1: The antibody and antigens used in this study

Organism	Antigen Name and Lot No	Antibody Name and Lot No
Hepatitis B	Core Antigen (HBcAg) (ab49014)	Anti-HBcAg antibody [14E11] (ab8638)
Hepatitis C	Core Antigen (ab49015)	Core antigen antibody [C7-50] (ab2740)
HIV1	p24 Antigen (ab43037)	Anti-p24 antibody [38/8.7.47] (ab9044)
T. pallidum	p47 antigen (ab51323)	Anti-p24 antibody (ab20923)

6.2.3.2 Biotin coated microbead experiments

MEMS chips surfaces are incubated with DSP (1mg/ml) overnight. They are rinsed with DMSO and PBS three times. Afterwards they are incubated with the streptavidin

antibody (4 $\mu\text{g/ml}$) for 3 hours and surface is functionalized for bonding of biotin coated microbeads with 0.01% w/v concentration.

6.2.4 Experimental Procedure

The cartridge containing parallel channels functionalized for different analytes was placed to the aluminum block temperature controller unit (Figure 6.5). The coordinates of each cantilever is identified in the Labview program with the help of a camera. The PBS solution is introduced to all channels and the temperature is set to 37°C. At first a frequency sweep is conducted at each channel and the drive frequency is defined. During this period, the antigen solutions were kept at 37°C on the site. The PBS solution in all channels was drained and the antigen solutions corresponding to each channel are introduced to the system. Also PBS solution is sent to the reference channel. The reference channel is not functionalized for any of the antigens. Also some negative control experiments were conducted by sending the antigen to wrong channels. During this process the phase difference between the coil and LDV output is monitored real-time. The phase shift data obtained from the experiments is saved in .xlsx file using the Labview interface. A polynomial is fitted to the data using Matlab and it is presented as the result.

6.3 Results

6.3.1 Microbead detection experiments

Before starting the measurements with the antigens the system is tested with microbeads which have higher mass. Figure 6.6 shows the result of the microbead experiment. The phase of each cantilever is normalized. At the beginning there was PBS in all the channels. The reference channel (5th Cantilever) has no surface functionalization. The channels 3 and 4 are also not functionalized whereas the cantilevers 1 and 2 are

functionalized with streptavidin. At first the microbead solution (0.01w/v) is introduced to every single channel. As expected a certain phase shift occurred in 1st and 2nd cantilevers due to binding. Also a smaller shift was observed in cantilever one which can be related with the non-specific binding. On the other hand no significant phase shift occurred in the reference cantilevers 3rd and 4th. In the 5th channel we also expected to not to observe any change, however probably due to non-specific binding a certain shift also occurred. After that the channels were rinsed with PBS for 3 times and phase stability was achieved again. This experiment shows the system is capable of mass detection by using parallel channels.

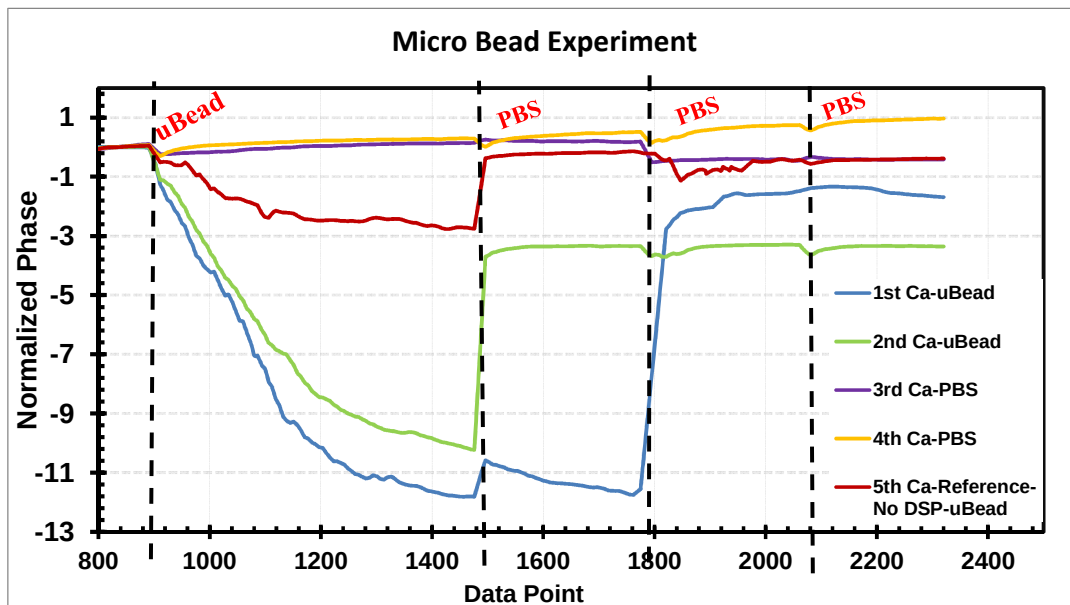
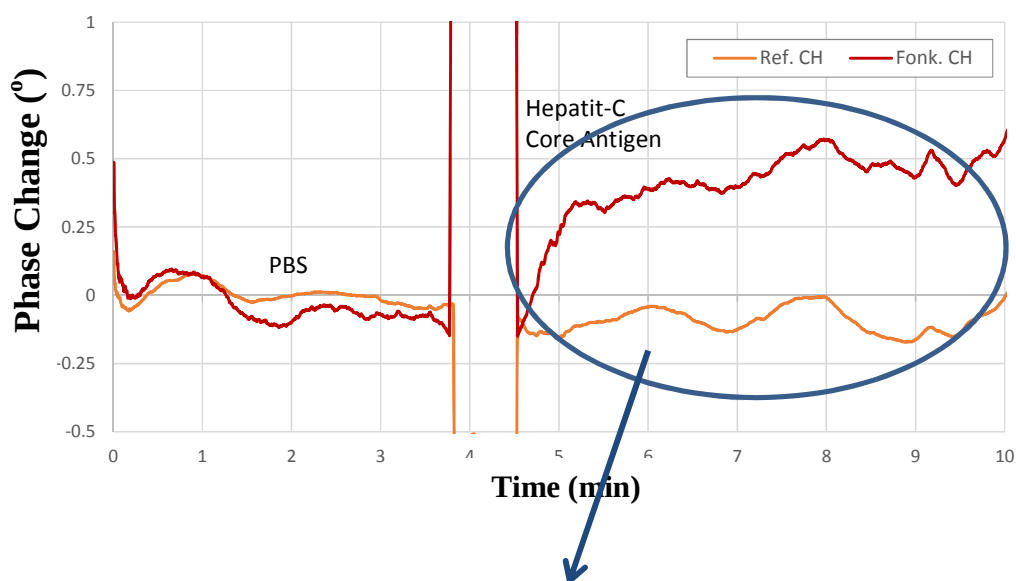


Figure 6.6: a) Micro bead experiment conducted in different channels.

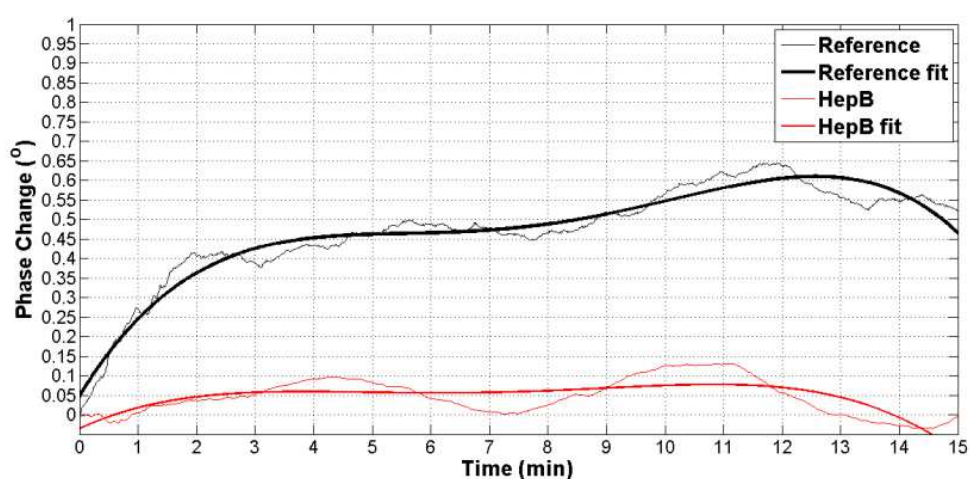
6.3.2 Immunoassay detection experiments

After the microbead experiments, the system is tested for detection of the specified antigens. The Hepatitis B (HBV) Hepatitis C (HCV), AIDS (HIV1) and syphilis bacteria (Tpallidum-Siphilis) are tested with the same scheme. The result of the experiment conducted with HCV antigen is shown in Figure 6.7a. The solution has a concentration of

1 μ g/ml. channel. In order to obtain the absolute change between the reference channel and the measured antigen, a polynomial is fitted to the raw data as shown in Figure 6.7b. The same procedure is applied for other types of antigens. There is phase difference of 0.45 $^{\circ}$ between the reference cantilever and the HCV bond cantilever.



a)



b)

Figure 6.7: a) The HCV experiment. b) The polynomial is fitted to the raw data.

Figure 6.8 shows the result of the experiment HBV experiment, again a 0.5° phase difference is observed due to binding.

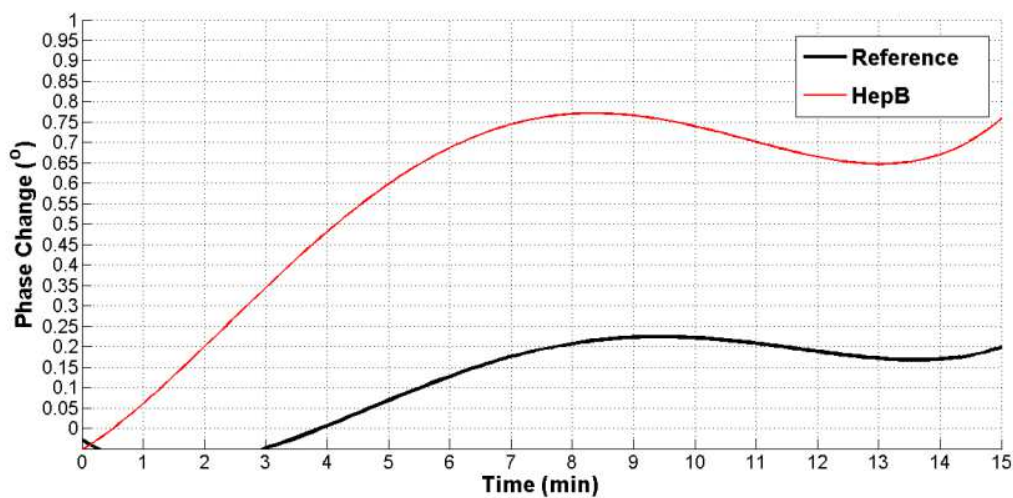


Figure 6.8: The HBV experiment

Figure 6.9 shows the result of the Syphilis experiment. This time the observed phase change is 0.05° . This difference may be due to the uncontrollable characteristics of the binding dynamics. The position of the bond proteins on the cantilever causes variations of the amount of frequency shifts. If the analytes bind to the tip of the cantilever it will cause the maximum shift. If they bind close to the anchor region it may even cause a stiffening effect and cause a negative shift [23, 73, 109]. After the syphilis experiment the HIV antigen is tested. The result of this experiment is shown in Figure 6.10. The phase difference between the HIV antibody coated cantilevers is 0.1° .

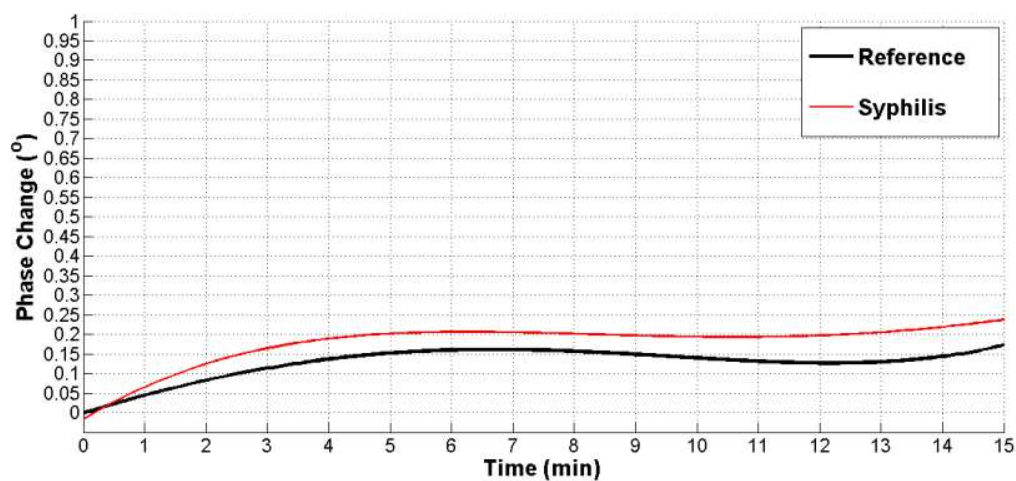


Figure 6.9: a) The Syphilis Experiment.

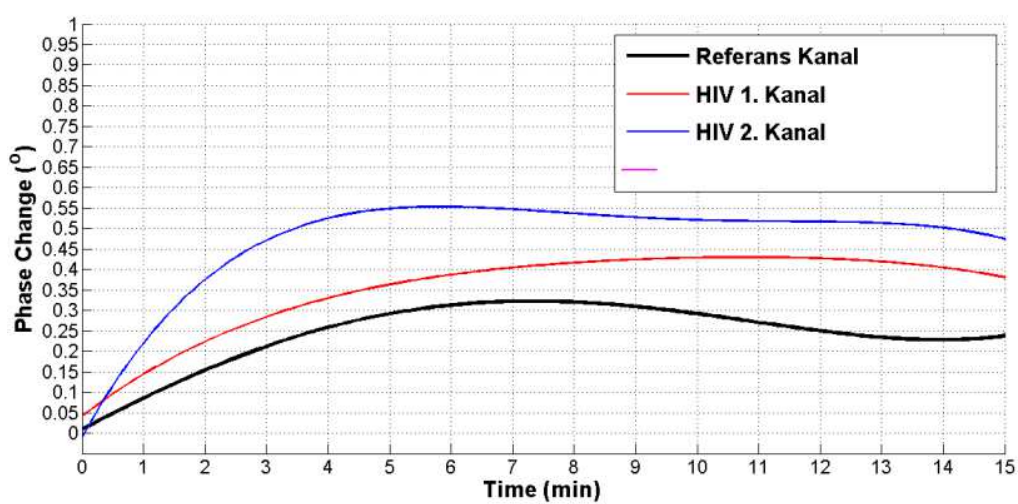


Figure 6.10: The HIV experiment.

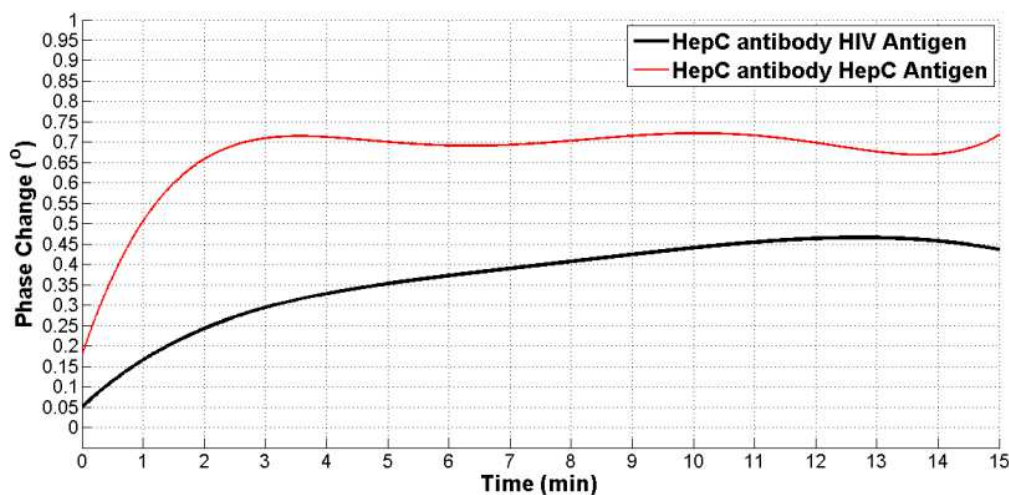


Figure 6.11: The negative control experiment with HCV antigen.

Finally a negative control test is conducted with the system. Two channels were functionalized with HCV antibodies. HCV antigen is introduced in one of them and HIV antigen is introduced to the other one. As expected no binding occurred between the HIV antigen and HCV antibody and that channel acted as a reference channel. This shows the selectivity of the method.

6.4 Conclusion

The system is tested for immunoassays from parallel channels. Detection in 5 channels was achieved with microbead experiments. In the functionalization process the cantilevers in some of the channels were broken and they couldn't function properly. Because of this results from two or three different channels are presented. Nevertheless the difference between the reference cantilever and the functionalized cantilever is observable. The noise floor of the system is 0.007° as reported in Figure 3.8. By using this value minimum detectable concentrations for HBV, HCV, HIV and syphilis are 20 ng/ml, 10ng/ml,

30ng/ml and 140 ng/ml, respectively. Sensitivity is between 100pM-1mM for all the solutions. According to the review papers in the literature [5, 13, 23] this value is on the edge of the limits for dynamic cantilevers operating at their first bending mode. The quality factor values in our system are between 2 and 5, which is parameter that limits the performance of the system. Also it should be mentioned that the repeatability of the results were generally poor in these tests. In addition to the best experimental results presented in this thesis, there were many failed experiments giving no meaningful results. This was mainly due to the uncontrollable characteristics of the biological binding sites and noise problems.

7 CONCLUSIONS AND FUTURE WORK

A microcantilever based biosensor is developed for different point-of care applications, liquid property monitoring and antigen detection purposes. The cantilevers are made of electroplated nickel and fabricated with a simple one-mask process. Also a second mask process is added to limit the binding sites for the antigen detection application. The microcantilevers are actuated by an external electro-coil near their resonance frequency. Either the diffraction grating based optical interferometric method or a laser Doppler Vibrometer is employed for the optical read-out. Both actuation and read-out are conducted remotely without requiring electrical connections to the cantilever and to the cartridge the cantilever is housed in. This feature enables the use of the technology as a point of care device with disposable cartridges. Two different methods are used as sensing principle. Either the shifts in the resonance frequency at constant phase is tracked with a phase-locked loop (PLL) or the system is excited at a constant frequency and the phase difference between the coil current and the optical sensor output is tracked with a Lock-in Amplifier.

A major portion of this thesis research was about the viscosity and the density detection using the proposed sensor system. The method is first utilized for blood plasma and serum viscosity detection. The blood plasma viscosity is an important parameter for diagnosis of many diseases and there is a need for miniaturized point-of-care devices for this application. A new protocol is developed for measurements with the PLL and the interferometric read-out setup. Using this method, viscosity measurement accuracies better than 6% is achieved in the range 0.86 and 3.02 mPa.s, which is a sufficient range to distinguish the healthy and unhealthy plasma. The sensitivity of the system is 0.03 mPa.s. Afterwards, the read-out and the sensing principle are altered and another protocol is developed for the LDV read-out and the Lock-in based phase tracking. The sensitivity and the dynamic range are enhanced to 0.01 mPa.s and 0.8 to 7.2 mPa.s, respectively. In both

studies the densities of the liquids were measured externally with laboratory balance. Subsequently, we deepened our work to develop a new method to detect both viscosity and density separately with microcantilevers. Although there are some studies in the literature dealing with this problem, they used frequency sweep based resonant frequency tracking, which is inherently slow. Instead of these methods we introduce a novel method utilizing two cantilevers with different widths to simultaneously measure viscosity and density. The accuracy of the density measurements is better than 3% for the range 997 to 1150 kg/m³. The accuracy of the viscosity measurements is better than 5% for the range 0.935 to 4 mPa.s. Based on the signal-to-noise ratio, the minimum detectable differences in the viscosity and the density are 1.6×10^{-3} mPa.s and 0.3 kg/m³, which is comparable [105] or better [69] than the state-of-the-art. Since we introduce a method, it is obvious that one can still employ our methodology to achieve higher accuracy and dynamic range by either choosing two cantilevers optimized for the intended range or by increasing the number of cantilever designs used in the measurement.

Another major part of this research was focused on adapting the proposed sensor system for blood plasma coagulation time measurements. Very detailed measurement protocols for coagulation time tests (PT and aPTT) are developed and repeatable results are obtained. The reliability of the system is tested with standard control plasmas. The percent coefficient of variation (CV %) for the tested samples are 7.28 and 6.33 for PT and aPTT measurements, respectively. These reproducibility values are comparable with the commercial devices available in the market [106, 156]. There is no statistical difference between our results and the data sheet of control samples used in the experiments. At the end of this part, aPTT and PT tests were successfully performed on the same cartridge simultaneously using plasma sample from a healthy donor. Protocols for functionalization and for different coagulation tests are developed.

Finally the systems capability for multiplexed antigen detection measurements is examined. Minimum detectable concentrations for HBV, HCV, HIV and syphilis are 20 ng/ml, 10ng/ml, 30ng/ml and 140 ng/ml, respectively. Minimum detectable concentration is between 100pM-1mM for all the solutions which are close to the detection limit for dynamic cantilevers operating at their first bending mode [5, 13, 23]. Repeatability was generally poor in our mass detection experiments due to various biological and environmental noise sources.

Future work for viscosity and density monitoring will focus on enhancing the sensitivity and the dynamic range using further optimized cantilever designs. Also the miniaturization of the system with a more compact optical read-out technique will be considered. Optical lever technique utilizing parallel read-out and fiber-based compact system are good alternatives. The measurements in multiplexed channels are conducted one at a time; they will be conducted simultaneously in the future with the modifications in the read-out. For coagulation time measurements the functionalization procedure will be optimized to attain standard mixing conditions and clinical tests will be conducted afterwards. Efforts are already underway for commercialization of the coagulation measurement system. The challenges associated with measurement using whole blood need to be addressed. .

PUBLICATIONS RESULTING FROM THIS THESIS RESEARCH

International Journal Papers:

- J1. **O. Cakmak**, N. Kilinc, E.Ermek, G.G. Yaralioglu, H. Urey “Real-Time Viscosity and Mass Density Sensor Based on Microcantilevers with Different Geometries” (under review)
- J2. **O. Cakmak**, N. Kilinc, E.Ermek, S. Bulut, I. Baris, I.H. Kavakli G.G. Yaralioglu, H. Urey “Cartridge Based Sensor Array Platform for Multiple Coagulation Measurements from Plasma” Lab on a Chip 15 (1), 113-120. (**featured as Hot Article**)
- J3. N Kilinc, **O Cakmak**, A Kosemen, E Ermek, S Ozturk, Y Yerli, ZZ Ozturk, H Urey “Fabrication of 1D ZnO nanostructures on MEMS cantilever for VOC sensor application” Sensors & Actuators B: Chemical, vol. 202, pp. 357-364, 2014.
- J4. **O. Cakmak**, C. Elbuken, E. Ermek, A. Mostafazadeh, I. Baris, B. E. Alaca, I. H. Kavakli, H.Urey “Microcantilever Based Disposable Viscosity Sensor for Serum and Blood Plasma Measurements,” Methods (63), 225-232, 2013. (**featured as Cover Article**)
- J5. **O. Cakmak**, C. Elbuken, E. Ermek, S. Bulut, Y. Kılinc, I. Baris, H. Kavakli, E. Alaca, H. Urey “MEMS biosensor for blood plasma viscosity measurements” New Biotechnology, 29, Supplement S162-S163, 2011. (also published as Proceedings of the European Congress on Biotechnology Conference)

International Conference Papers:

- C1. **O. Cakmak**, E.Ermek, N. Kilinc, I. Baris, I.H. Kavakli, G.G. Yaralioglu, H. Urey “Microcantilever based LoC System for Coagulation Measurements” accepted to The 18th International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTas 2014), San Antonio, Texas, USA, October 2014.
- C2. U. Baran, S. Holmstrom, D. Brown, W. Davis, **O. Cakmak**, H. Urey, “Resonant PZT MEMS scanners with integrated angle sensors” IEEE/LEOS International Conference on Optical MEMS and Nanophotonics, Glasgow, Scotland, August 2014.
- C3. **O. Cakmak**, N. Kilinc, E.Ermek, G.G. Yaralioglu, H. Urey “MEMS Sensor for Blood Plasma Viscosity and Coagulation Time Measurements” 11th Int. Workshop on Nanomechanical Sensing (NMC 2014), Madrid, Spain, May 2014.
- C4. **O. Cakmak**, N. Kilinc, E.Ermek, A. Mostafazadeh, C. Elbuken, G.G. Yaralioglu, H. Urey “LoC Sensor Array Platform for Real-Time Coagulation Measurements ” IEEE MEMS 2014, San Francisco, California, USA, January 2014.
- C5. **O. Cakmak**, N. Kilinc, E.Ermek, G.G. Yaralioglu, H. Urey “MEMS Based Blood Plasma Viscosity Sensor Without Electrical Connections” IEEE Sensors 2013, Baltimore, Maryland, USA, November 2013.
- C6. N Kilinc, **O Cakmak**, A Kosemen, E Ermek, S Ozturk, Y Yerli, ZZ Ozturk, H Urey, “A Voc Sensor Based on Micromechanical Cantilever Functionalized with ZnO Nanorods” The 17th International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTas 2013), Freiburg, Germany, October 2013.
- C7. **O. Cakmak**, C. Elbuken, E. Ermek, S. Bulut, Y. Kilinc, I. Baris, H. Kavakli, E. Alaca, H. Urey, ”Mems Biosensor For Blood Plasma Viscosity Measurements”, European Congress on Biotechnology, Istanbul, Turkey, September 2012.

APPENDIX: MATLAB CODES FOR COAGULATION CURVE FITTING

Polynomial Fit Routine:

```
function [t,y,tinterval,dinterval]=polinom (data,data1,p)
x1=data1(:,p(1)); %recall time data
y1=data1(:,p(2)); %recall phase data
N=12 %order of poly

xint=data(:,p(1)); %time data
yint=data(:,p(2)); %phase data
P=polyfit(xint,yint,N); %polyfit
t = linspace(min(xint),max(xint),length(xint)); %reorganize time

%Put polynom coefficients to generate the array for specific interval

y=zeros(1,length(t));
for i = 1:N+1
    y = cat(1,y ,t.^(N+1-i)); %put poly coeffs into equation
end
y = y(2:N+2,:);

y = P*y;
z=length(y1)-length(y);
k=zeros(1,z);
y=cat(2,k,y); %kind of zero padding the nonfitted parts
y(1:z)=y1(1:z);
t=linspace(min(x1),max(x1),length(x1));%reorganize time

d=3*diff(y,1);%first derivative
[l,I]=min(d);
dinterval=d(I-8:1:I+6); %derivative plot interval
tinterval=t((I+1)-8:1:(I+1)+6); %time plot interval
```

Plotting the coagulation curves of different repetitions:

```
clc
clear all
close all
```

```
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%NORMAL PLOTS%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
```

```
data=xlsread('plot_data.xlsx','Normal', 'a17:f50'); %import phase and
time data (this includes all the repetations)
data1=xlsread('plot_data.xlsx','Normal', 'a3:f50'); %import phase and
time for a chosen interval data (this includes all the repetations)
```

```
p=[1;2] %use 1st repetation's phase and time
[t,y,tinterval,dinterval]=polinom(data, data1,p)
%polynomial fit to the chosen interval and take first derivative
```

```
hold on
subplot (2,1,1);%plot fitted curve
plot(t,y,'r-', 'lineWidth', 1.5, 'LineSmoothing', 'on')
grid on
hold on
subplot (2,1,2); %plot 1st derivative of the chosen interval
plot(tinterval,dinterval,'r-', 'lineWidth', 1.5, 'LineSmoothing', 'on')
grid on
```

```
%
%
```

```
p=[3;4] %use 2nd repetation's phase and time
[t,y,tinterval,dinterval]=polinom(data, data1,p)
%polynomial fit to the chosen interval and take first derivative
```

```
hold on
subplot (2,1,1);%plot fitted curve
plot(t,y,'r-', 'lineWidth', 1.5, 'LineSmoothing', 'on')
grid on
hold on
subplot (2,1,2);%plot 1st derivative of the chosen interval
plot(tinterval,dinterval,'r-', 'lineWidth', 1.5, 'LineSmoothing', 'on')
grid on
```

```
%
%
```

```
p=[5;6]%use 3rd repetation's phase and time
[t,y,tinterval,dinterval]=polinom(data, data1,p)
%polynomial fit to the chosen interval and take first derivative
```

```

hold on
subplot (2,1,1);%plot fitted curve
plot(t,y,'r-', 'lineWidth', 1.5, 'LineSmoothing', 'on')
grid on
hold on
subplot (2,1,2);%plot 1st derivative of the chosen interval
plot(tinterval,dinterval,'r-', 'lineWidth', 1.5, 'LineSmoothing', 'on')
grid on

%
%
%
% %%%%%%%%%%%%%%ABNORMAL1
% PLOTS%%%%%%%%%%%%%
%
%
%
data=xlsread('plot_data.xlsx','Abnormal1', 'a16:f60');
data1=xlsread('plot_data.xlsx','Abnormal1', 'a3:f60');

p=[1;2]
[t,y,tinterval,dinterval]=polinom(data, data1,p)

hold on
subplot (2,1,1);
plot(t,y,'g--', 'lineWidth', 1.5, 'LineSmoothing', 'on')
grid on
hold on
subplot (2,1,2);
plot(tinterval,dinterval,'g--', 'lineWidth', 1.5, 'LineSmoothing', 'on')
grid on

%
%

p=[3;4]
[t,y,tinterval,dinterval]=polinom(data, data1,p)

hold on
subplot (2,1,1);
plot(t,y,'g--', 'lineWidth', 1.5, 'LineSmoothing', 'on')
grid on
hold on
subplot (2,1,2);
plot(tinterval,dinterval,'g--', 'lineWidth', 1.5, 'LineSmoothing', 'on')
grid on

```

```

%
%

p=[5;6]
[t,y,tinterval,dinterval]=polinom(data, data1,p)

hold on
subplot (2,1,1);
plot(t,y,'g--','linewidth', 1.5, 'LineSmoothing', 'on')
grid on
hold on
subplot (2,1,2);
plot(tinterval,dinterval,'g--','linewidth', 1.5, 'LineSmoothing', 'on')
grid on

%
%
% %
% %%%%%%%%%%%%%%ABNORMAL2
% PLOTS%%%%%%%%%%%%%
%
%
data=xlsread('plot_data.xlsx','Abnormal2','a46:f70');
data1=xlsread('plot_data.xlsx','Abnormal2','a3:f70');

p=[1;2]
[t,y,tinterval,dinterval]=polinom(data, data1,p)

hold on
subplot (2,1,1);
plot(t,y,'b-.','linewidth', 1.5, 'LineSmoothing', 'on')
grid on
hold on
subplot (2,1,2);
plot(tinterval,dinterval,'b-.','linewidth', 1.5, 'LineSmoothing', 'on')
grid on

%
%

p=[3;4]
[t,y,tinterval,dinterval]=polinom(data, data1,p)

hold on
subplot (2,1,1);

```

```

plot(t,y,'b-.', 'linewidth', 1.5, 'LineSmoothing', 'on')
grid on
hold on
subplot (2,1,2);
plot(tinterval,dinterval,'b-.','linewidth', 1.5, 'LineSmoothing', 'on')
grid on

%
%

p=[5;6]
[t,y,tinterval,dinterval]=polinom(data, data1,p)
hold on
subplot (2,1,1);
plot(t,y,'b-.', 'linewidth', 1.5, 'LineSmoothing', 'on')
grid on
hold on
subplot (2,1,2);
plot(tinterval,dinterval,'b-.','linewidth', 1.5, 'LineSmoothing', 'on')
grid on

hold on

% %%%%%%%%%%Define Plot
Properties%%%%%%%%%
subplot (2,1,1)
xlabel('Time(s)'); ylabel ('Phase Change (^o)')
axis([0 max(t) -11 2])
legend('Normal Control Plasma', 'Abnormal1 Control Plasma', 'Abnormal2
Control Plasma', 'Location','NorthEast');
set (gca, 'FontSize', 14)
figureHandle = gcf;
%# make all text in the figure to size 14 and bold
set(findall(figureHandle,'type','text'),'fontSize',16,'fontWeight','bold'
,'fontName', 'arial')
set(gca,'xtick',[0:5:max(t)])
set(gca,'ytick', [-40:2:15])
title('PT Tests with Control Plasmas')

subplot (2,1,2)
xlabel('Time(s)'); ylabel ('(d(^o)/dt)')
axis([0 max(t) -6 2])
legend('Normal Control Plasma', 'Abnormal1 Control Plasma', 'Abnormal2
Control Plasma', 'Location','NorthEast');
set (gca, 'FontSize', 14)
figureHandle = gcf;
%# make all text in the figure to size 14 and bold

```

```
set(findall(figureHandle, 'type', 'text'), 'fontSize', 16, 'fontWeight', 'bold',  
    'fontName', 'arial')  
set(gca, 'xtick', [0:5:max(t)])  
set(gca, 'ytick', [-40:1:15])  
title('First Derivatives')
```

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