Flow Force Augmented 3D Suspended Polymeric Microfluidic ($SPMF^3$) Platform for Sensitive Diagnosis

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Abstract

Flow Force Augmented 3D Suspended Polymeric Microfluidic ($SPMF^3$) Platform for Sensitive Diagnosis

Mostapha Marzban, PhD of Mechanical Engineering
Concordia University, March 2017

A 3D suspended polymeric microfluidics ($SPMF^3$) for sensitive diagnosis is reported in this study. Design, simulation, fabrication and experimental validations for different applications are presented. Using this innovative lab on a chip platform, variety of biophysical measurements can be done on bioparticles and cells such as detection, counting, flexibility and sizing without any external excitation.

Study of cells and bioparticles have been one of the main foci of microsystems due to their advantages such as simplicity, low price and portability. These microsystems can be categorized in two main groups based on their design which are either microcantilevers or micofluidics. Biodiagnostic microsystems employ different transduction principles to study cells and bioparticles. Microcantilevers work based on mechanical principles such as deflection or frequency variation. However, Microfluidics work based on optical and electrical techniques such as light detection and impedance variation.

Microcantilever systems have shown a broad range of cells and bioparticles detection with a relatively low analysis time in comparison with other methods. This has brought more focus and studies towards this technique. The most innovative microsystem for biophysical study is Suspended Microchannel Resonator (SMR) which has overcome the main issue of microcantilever based systems that have low quality factor imposed by damping effect of surrounding liquid medium.

The first SMR was micromachined in silicon wafer and was actuated using electrostatic excitation electrodes. The microcantilever movements applied by external excitation was captured by an optical laser method. It means that bioparticles passing through suspended microchannel with a weight in the order of nanogram cannot deflect the highly stiff silicon microcantilever. To overcome this issue, new SMR suspended microfluidics has been investigated which is made of Polydimethylsiloxane (PDMS). This innovation has decreased microchannel stiffness enough to detect bioparticles. In other words, the necessity of integration of external excitation electrodes has been removed by using PDMS instead of silicon material.

In this study, a 3D suspended polymeric microfluidic platform was designed and fabricated for sensitive biodiagnostic applications. To improve the sensitivity of the former suspended microfluidic systems, microchannel plane was modified from microcantilever plane by 90 degrees. This innovative design was simulated and fabricated to validate the concept. The finite element modeling shows that the $SPMF^3$ platform is five times more sensitive in comparison with the other suspended microchannel concepts. Detection of variations in fluid properties which results in diagnosis of bioelements modification during a process has been completely done using the $SPMF^3$. It is shown in this thesis that kinematic viscosity is the only fluid parameter that can be used to monitor the variations of fluid properties. Finally, detection and diagnosis of bioelements have been performed and validated using polystyrene microbeads and air bubbles inside the suspended microchannel. In this experiment, 60µm-polystyrene beads were detected using the $SPMF^3$. Moreover, different air bubbles with multiple dimensions and flow rates were detected and studied while passing through the suspended microfluidics. The sensitivity of $SPMF^3$ platform to microparticles can be increased by eighteen times according to the finite element modeling results which is done with varying micro-nozzle dimensions.

This thesis also presents a simple theoretical model along with finite element analysis and experimental validation on the effect of fluid properties of both Newtonian and non-Newtonian fluids on the static behavior of suspended 3D microfluidic platforms.

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A long journey always looks short for people from outside of it however, every single up and down moments touched me all the way to my bones. Shocking moments of my illness when I never expect such a problem which followed by a period of heavy rain fall of issues in my life never stopped me thinking of what I want from this lifespan.

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Dedicated as an appreciation of limitless love and support

To:

My wife, Dina

My parents, Ommolbanin and Naser

Table of contents

List of Figures

List of Tables

List of Symbols

A	cross section area
E	Modulus of elasticity
f_1	Applied flow force at point 1
f_2	Applied flow force at point 1
k_m	Microcantilever mechanical stiffness
$\dot{m}$	Mass flow rate
P_in	Flow pressure at nozzle inlet
P_out	Flow pressure at nozzle outlet
t	time
Q	Volume flow rate
V_in	Flow velocity at nozzle inlet
V_out	Flow velocity at nozzle outlet
V_b	Flow velocity at point b
V_c	Flow velocity at point c
w	Nozzle width
ρ	Density
μ	Dynamic viscosity
[μ]	Intrinsic dynamic viscosity
$[\upsilon]$	Intrinsic kinematic viscosity
υ	Kinematic viscosity
δ	Microcantilever deflection
ω	Oscillation frequency
ω_{vac}	Frequency response in vacuum
Δp	Pressure difference between two marks
ΔV	Velocity difference between two marks

Nomenclature

3D	Three-Dimensional
CAD	Computer-Aided Design
FEA	Finite Element Analysis
FOB	Flow plane Orthogonal to Bending plane
FSI	Fluid Structure Interaction
MEMS	Micro Electro Mechanical Systems
PDMS	Polydimethylsiloxane
SMR	Suspended Microchannel Resonator
SPMF	Suspended Polymeric Microfluidics
$SPMF^3$	3D Suspended Polymeric Microfluidics
SU8	Negative Photoresist
UV	Ultraviolet

Chapter 1

Introduction and Literature Review

1.1. Introduction

Design and fabrication of a 3D suspended microfluidic system which is capable of bioelements detection and measuring variations in their biophysical properties is the main objective of this study. The main parameters of bioparticles are such as flexibility, weight, count and size of them. The 3D suspended polymeric microfluidics ($SPMF^3$) detects biomechanical properties through deflections of the suspended microchannel. Depending on the parameter under investigation, this deflection may be due to flexibility, mass flow rate or dimension of the bioelements. Moreover, the effect of bioparticles flow inside suspended microchannel will be intensified due to the innovative design of the 3D microfluidic channel which results in more deflection and consequently higher sensitivity.

Biologists have been looking for cell physical parameters since the first microscopic pictures made this area visible. Since then, investigating cell mechanobiological properties were considered seriously. Mechanobiological properties of cells are such as how cells are detecting a force and how they react to these external excitations. These forces will define the biological processes of a cell such as its growth rate. Thus, defining a function between the amount of applied force and reaction of cell has been one of the main goals in biology that has come to reality using Micro-Electro-Mechanical Systems (MEMS). One of the key microsystems to perform such measurements are microcantilevers which have gathered a high amount of interest because of their capabilities in accurate cells manipulation, measurement of applied forces to cells and the cellular force reactions. These microstructures can be made out of silicon or polymers depending on the application.

Mechanical biodiagnostic systems have shown high capability in cell studies due to their simplicity and their ability to be microfabricated which is necessary to manipulate cells. Using these microcantilever systems, the microparticles of femtogram mass have been detected. Figure 1-1 shows the capabilities of mechanical microsystems in comparison with their optical and electrical competitors. As it is shown in this reference [1], limit of detection in mechanical systems has been well distributed versus analysis time. In other words, an appropriate mechanical microstructure can be employed, based on the required detection time. This feature as well as other advantages have made these microsystems a desirable mechanobiological study tool.

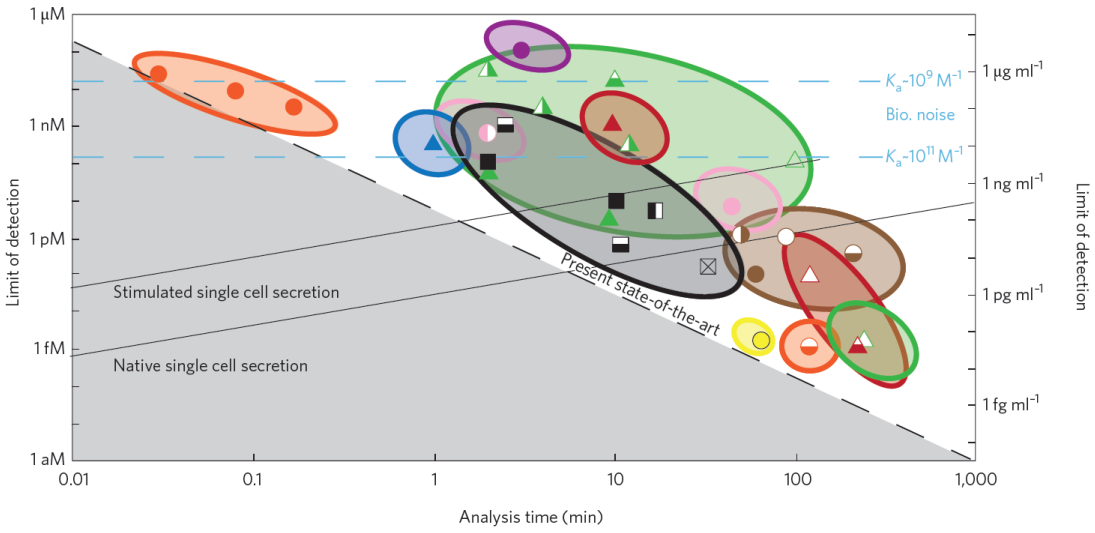


Figure 1-1. Limit of detection versus time for different microsystems (○ Opticals, △ Mechanicals, □ Electricals) [1]

Another important biophysical parameter of cells is their number and detecting different or malignant ones [2]. Thus, detecting and counting them in some biological experiments is so crucial. Number of cells in a living body projects plenty of information about its health. To keep a body or a tissue alive, nutrition should be transported frequently. To perform these assignments, different cells should be employed. The number of these cells have to be always in a limit of balance. On the other hand, when a body is under medication, it means that number of malignant cells should be reduced by certain medications. In other words, monitoring number of cells is an important item in development of new medications.

Commercial cytometry machines in biological labs are currently performing cell detection, counting and sorting processes which are expensive, the process needs high amount of sample, sample preparation and post processing by specialists [3]. On the other hand, using microsystem developed for biological and chemical processes are cheap, portable and user-friendly microchips that are able to implement biological lab experiments. As an example, Lancaster et al. [4] showed the feasibility of a credit card size cell counter which is able to do all experiment processes within a single chip and less complicated.

Counting, sensing and mechanobiological measurements of cells using some small and portable devices can drastically contribute to human health care. This would forecast major illnesses which can be easily cured if diagnosed in early stages. Sensing biomaterials and chemicals have been in the focus of MEMS technology due to its inherent advantages. These microsystems are cheap, tiny and they have shorter processing time and use smaller amount of samples.

For studying biophysical properties of cells and microparticles the first step is how to detect them. Detection method categorizes these microsystems into two main classes of microcantilevers and microfluidics in which there are three main transduction techniques, such as mechanical (deflection and frequency based), optical and electrical [5]. Based on the type of microsystem and the bioelement under study, different transduction principles will be employed as shown in this flowchart (Figure 1-2).

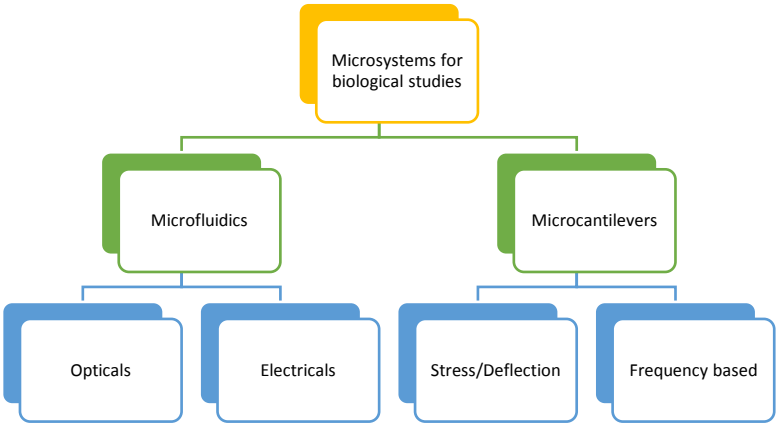


Figure 1-2. Flowchart of microsystems which are mostly used for biological studies

In this study, first a brief review of cell biophysical analysis and mechanobiological study using different microsystems such as microfluidics and microcantilevers is presented. Then, our proposed SPMF^3 platform which will act as a lab on a chip and can detect and study bioelements with high sensitivity is presented. Finally, the work outline of this thesis is summarized.

1.2. Cell biophysical study using microsystems

Microsystems have gathered great interests and shown valuable results in the study of mechanobiology and biophysical analysis of cells due to their properties such as: microsystems match cell dimensions, provide growth microenvironment such as in-vivo environment, facilitate parallel analysis that can be done on cells through integration of other sensors or chemicals to the microsystem, low cost, low amount of sample consumption, etc. There are a variety of microsystems that provide us measuring cell specifications such as physical, mechanical and chemical. In this literature review, these microsystems have been categorized into two divisions, Microfluidics and Microcantilever based diagnostics.

1.2.1. Microfluidics based diagnostics

Cells biophysical properties such as mass, size, number, etc. have been the subject of many studies. The main methods to investigate cells using microfluidics are: Microfluidic resistive pulse technique, capacitance counters and optical methods. To extract cell biophysical properties, cell counting machines have provided an appropriate infrastructure for this goal. In these micromachines, first cells are detected which is required in any cell study. Then, cells can be sorted and sized based on the measured data. Thus, this kind of cell analysis microsystems has been considered in our study as a proper tool for extraction of cell biophysical properties.

Counting cells in a sample had been one of the most important parameters in medical and biological researches due to its wide applications. Before Coulter innovation in 1953 [3], all the counting processes were some time-consuming processes of looking and counting cells under a microscope. Since then this measurement has been conducted in an advanced and less exhausting way. Fluorescence based cytometry was first introduced by Gohde in 1968

which was absorbed by medical industries soon. Commercialized and industrial fluorescence based flow cytometers were introduced in few years. To name few companies that contributed a lot to flow cytometry, one can say Beckman-Coulter and BD-bioscience though, there are many other companies that work on their cell analysis devices. Although these companies' devices were addressed biologists needs, cytometry devices are bulky, consume high sample amounts and expensive.

A) Microfluidic resistive pulse method

This method is based on Coulter innovation which is applied in microfluidic chips. W. H. Coulter connected two fluid containers through a pore in which there is an electrode in each fluid container (Figure 1-3). If the fluids are electronically conductive when particles pass through the aperture an electronic pulse is generated. This pulse is produced since particles such as cells are mainly nonconductive. This signal can be impedance, conductance or reflected frequency power [3]. Today, lab instruments which work based on Coulter method are capable of measuring number, size and surface area of microparticles from 0.4μm. Although these devices made biomolecule and microparticle inspection an easy task, these instruments are still bulky and expensive. To make some portable, small and low cost devices it is required to apply micromanufacturing techniques to the Coulter method.

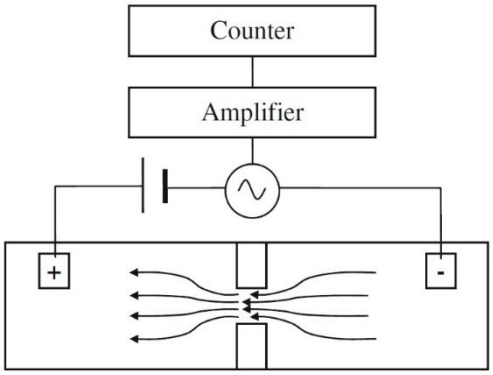


Figure 1-3. Coulter counter, illustration of its parts [3]

Resistive pulse method has been employed for counting and detection of cells or particles. As the microfabrication method is advancing, more microstructures based on resistive pulse methods have been investigated and introduced. In 1970, DeBlois et al [6] designed and tested a microsystem to detect nanoparticles of polystyrene with a minimum size of 90 nm.

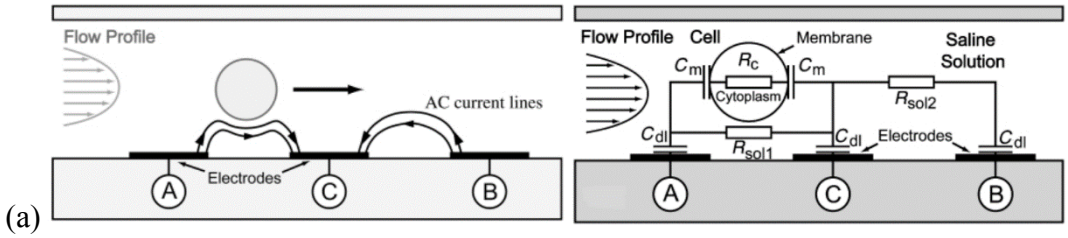
To conduct a fluid in a microchannel, two mostly used methods are electrokinetic and hydraulic pressure flow. Main disadvantages of this method are low throughput and detection sensitivity. In other words, the flow rate in microsystem which is sensitive to microfluidic channel size limits the throughput. And, detection sensitivity which is derived by volumetric ratio of detectable particle size to microchannel width or pore size limits detectable particle sizes. This means that sensitivity and throughput are two parameters which are against each other and improving one results in degrading the other.

With decreasing aperture diameter and/or electronic noise reduction, one can detect and count smaller particles even though, particles flow rate will be affected. In DeBlois experiment, the sensitivity of their system was about 0.06%. This means the volumetric ratio of the detected 90nm polystyrene particles to the microchannel pore was 0.06%. Using the better electrical circuits, the more signal to noise ratio amounts were achieved in the later studies. One of the most noted investigations which has gained the lowest volumetric ratio is done by Wu et al. in 2008 [7]. They have detected 520nm polystyrene particle through a 20 μm pore. The sensitivity of their system was 0.0004% which is ten times lower than the sensitivity of a commercial Coulter device. To obtain this sensitivity they have employed a pulse resistive as well as a fluorescence detection systems. This integration has drastically increased the signal to noise ratio. However, small channel size and detectability criteria have limited the actual throughput of the microsystem to 30 particles/min.

Regarding low throughput issue, several investigators tried to use multichannel systems to increase sample flow rate and decrease experiment duration. In 2007, Zhe et al. [8] designed and tested a multichannel counter with higher throughput which is able to count particles three times faster than a single channel system. Impedance or restive based microsystems initiated based on Coulter innovation. However, performance/sensitivity of the system was dependent on aperture size. In other words, to detect and count smaller cells, smaller pores are required. And on the other hand, by shrinking the pore size, flow rate of microstructure would drop. Since bioparticles are in very dilute concentrations, reducing aperture size consequently means longer processing time to count all of the cells within a sample.

In 2001, Gawad et al. [9] proposed a new breakthrough in this type of systems to address this issue to some extent. They designed and tested a microfluidic system in which the

electrodes are embedded in the side walls of microchannel (Figure 1-4). In their first try, a microfluidic channel with two pairs of electrodes embedded on the bottom side of its channel designed and tested. When a cell/particle passes through the channel, electric field between adjacent electrodes are deformed. This change in electric field generates a pulse in impedance signal which is proportional to particle characteristics. This innovation provided clear discrimination between beads and cell during experiments.



(a) (b)

Figure 1-4. Sensing process in a microchannel in which electrodes are embedded on walls; moving particle deform electric field (a), representative electrical circuit (b)

Besides, they reached a high throughput of 100 cell s^{-1} using a 20×20µm channel which was the highest throughput at its time in impedance cytometers. This new technique was adopted and employed in many studies afterward. In one of the most noted ones, Wood et al. [10], showed a very high throughput of 25000 bead s^{-1} for counting of 15µm polystyrene beads. To this end, a radiofrequency probe to measure reflected rf power was employed. They used PDMS material in which a channel with size of 200×40µm is embedded. The main drawback of their system is that the size discrimination was not detectable from impedance results. This problem comes from the non-homogenous electric field which is due to employment of planar electrodes in the microchannel.

This issue was addressed by defining a new parameter called opacity [11]. This parameter depicts the ratio of high frequency impedance data to low frequency one. Using this parameter, Cheung et al. [12] could discriminate the differences between red blood cells (RBC) and ghost cells. However, to reach this discrimination ability they could not keep throughput of their microsystem and it was reduced to 100 cell s^{-1}.

Former investigations show that cells can be sized, differentiated and counted at impedance signal frequencies of around 500kHz. In higher frequencies up to orders of 1-10MHz, cell membrane capacitance affects the results. And in the frequency range of 20-100MHz, the

status of cell cytoplasm dominates the results [13]. This will aid biologist to go further inside the cells for investigations.

So far all the impedance counter systems were able to give just an average number of cells. To overcome this issue Gawad et al. [14] introduced a new method to get more accurate results. They employed a broadband impedance spectroscopy method for single cell counting in a short duration of 1ms. In this method, they used pseudorandom white noise as stimulating signal instead of regular sinusoidal signal. However, using pseudorandom white noise as stimulating signal degrades the signal to noise ratio which reduces the sensitivity of the microsystem. This issue was later addressed using adaptive filtering techniques by Sun et al. [15].

To increase the sensitivity of this method, other approaches have been proposed and tested. For example, moving particles toward electrodes will increase the signal to noise ratio as signal strength is higher near electrodes. To this end, Rodriguez et al. [16] used laminar flow benefits in microchannels. They have used a sheath to concentrate particles flow towards the electrodes. Using this microsystem they could count 20µm latex beads with a flow rate of 20 particles/s inside a microchannel with size of 190×50µm. To adjust the particles in two dimensions within the channel, increase the sensitivity of system and flow rate, Rodriguez et al. [17] improved their design (Figure 1-5). Using modified microfluidics with 2-D focusing sheath flow they could count 20µm polystyrene particles with flow rate of 1000 particles/s inside a channel with a size of 180×65µm.

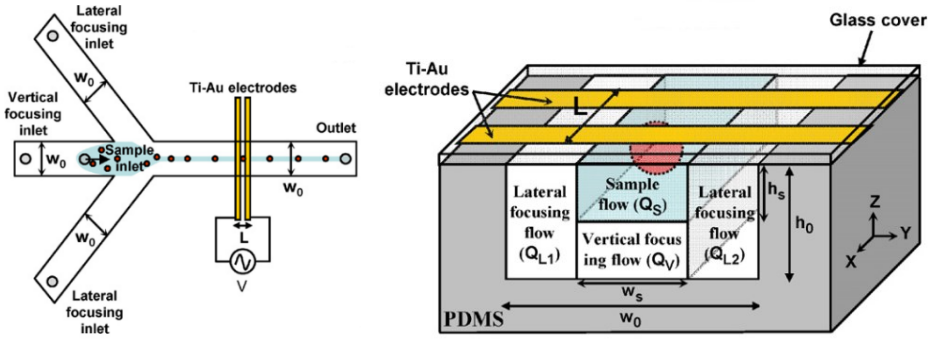


Figure 1-5. Particle counter with 2-D focusing sheath flow [17]

In this study, sheath flow has concentrated particles in an area of 32×25µm. Similarly, they could count 5µm yeast cells inside a channel with size of 100×43µm. Without using particle

concentration method by sheath flow, the microparticles were not even detectable in such a microchannel.

Using insulating sheath fluid such as oil, Bernabini et al. [18] have shown that current density inside the particles flow will be increased. This will improve signal strength and reduce current wastes through sheath flow. To employ this advantage, they used a conductive fluid for particles flow and an insulating oil for sheath flow. In their experiment, they could count and detect polystyrene beads of 1 and 2μm as well as E. Coli bacteria inside a 200×30μm channel. Since the channel cross sectional area is not confined in this method to detect smaller particles, the sensitivity value is around 0.007% which is very high in such microsystems.

B) Capacitance method

The capacitance principle as it is clear from its name, measures any change in electrical capacity between two electrodes which are attached to microchannel sides. Besides, it is very similar to resistive pulse method with the only difference in measured parameter. This type of cell analysis microstructure has been in interest of many research teams. Applications such as particle identification, cell cycle analysis and DNA content monitoring are among most noted applications of capacitance based microsystems. In one of the few examples of cytometry using capacitance method, Sohn et al. [19] designed and tested a capacitance microsystem to count bioparticles. Using this, they could count cells with a flow rate of 1μl/hr. This low flow rate proves that why this method has not been employed for cell counting in comparison with its applications in DNA content analysis. This method may also be employed for investigations on organic or inorganic particles which can be polarized.

C) Optical methods

Detecting reflected light or absorbed light when light hits a particle are two commonly used methods to detect microparticles. It is apparent that distinction based on detection of reflected light is easier and more sensitive than detection of absorbed light. It looks such as detection of light in darkness or detection of dark spots in light. However, more sensitive detectors are required for detection of scattered light which is more expensive than the other detectors used for absorbed light detection. Measuring scattered light or blocked light are used in particle counting application based on their ease and usefulness. For example, light

reflection detection is mostly used for counting microparticles in Air. And, light absorption detection is used for counting biomolecules in water or fluid.

This method has shown the capability of measuring cells and particles greater than 2μm which is enough to detect and count blood cells. For instance, Pamme et al. [3] employed laser light scattering method with two different positions for detection of scattered light. Using appropriate optical devices such as lenses to increase efficiency of the microsystem, they could detect and count particles of 2-9μm [20]. Regarding particle discrimination and sorting, the microsystem showed difference between particles with a diameter ratio of 1:2. This sensitivity can be improved, if better signal noise reduction methods employed.

Light blocking detection method was also employed to size and count bioparticles. Xiang et al. [21] designed a microstructure based on two pairs of light source and detection fibers. They tested their system to count and size particles of 10, 20 and 25 μm. When particles are passing in front of light source fiber, the amount of received light is decreased in light detection fiber. This light detection pulse delivers two sets of information. First, number of passing particles and second, the size of particle by analyzing the signal amplitude. The second set of optical fibers has been employed to measure particles velocity. By comparing time difference between two optical signals, one can determine particles velocity. To improve its sensitivity, their main challenge was how to perfectly align input and detection fibers which is a tough job in real essays.

To make a flow cytometer, Kummrow et al. [22] designed a microfluidics to count and detect cells with integrating both optical and impedance method in one system (Figure 1-6). First, to increase the sensitivity, they employed four optical fibers to detect scattered light and loss light. After this optical module, cells were passed through a gap between two electrodes to measure impedance variations.

Passing cells through this modulated flow cytometer, they obtained better signal to noise ratio and could measure cells larger than 2μm [22]. To perform their experiment, blood samples were taken from volunteers. Red blood cells (RBC) and with blood cells (WBC) concentration in human blood are $4\text{-}5.7\times10^{6}$ μl^{-1} and $3.5\text{-}10\times10^{3}$ μl^{-1} respectively. However, to count these cells using a microsystem, the sample should be diluted depending on the throughput of microfluidic system. In this experiment, Kummrow et al. [22] diluted WBC

sample to 500 cells/µl and flowed with a flow rate of 5µl/min that took 24 hours to be fully processed.

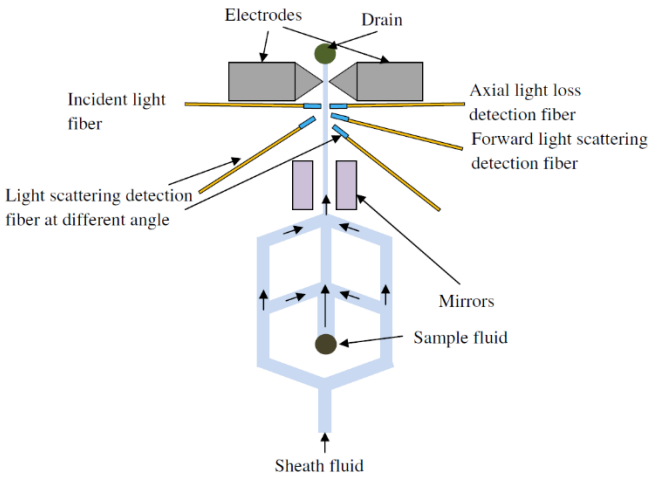


Figure 1-6. Schematic view of flow cytometer with optical and electrical modules [3]

McClain et al. [23] also used optical method for detection and counting of E. Coli cells. They used four microchannels in which the flow is transported using electrokinetics; they labeled the bacteria using fluorescence technique. This integration of scattering and fluorescence detection enabled them to identify cell size and nucleic acid content as well as count of them with a throughput of 35-80 cells s^{-1}. To increase throughput, Tung et al. [24] extended microchannel width (detection area). This lets them increase its flow rate to 500 cells s^{-1}. To this end, they increased the cross session of detection channel from 20×50µm in McClain's work to 100×300µm. However, to keep the detectability/sensitivity of their microsystem, they employed multi-color excitation and fluorescence detection. Besides, they used an amplification technique to increase the signal to noise ratio.

To use the advantages of bigger microchannels while keeping the sensitivity, another option is to employ sheath flow for focusing particles in the center of the microchannel. Mao et al. [25] designed a microstructure with 3D focusing technique by Dean vortex flow in a curved microchannel. Using a 90deg bend in the microchannel would provide Dean vortex flow that imposes a momentum by which particles are focused vertically in the middle of the channel. Applying other sheath flows from 2 other ports will align particle on a straight line in the center of the microchannel. The aforementioned technique was integrated with fluorescence detection system on a 100µm channel. Two fluorescent polystyrene particle with different

sizes of 7.32μm and 8.32μm were used to verify its performance in particle focusing and cytometry. Using this microsystem, they reached to a flow of 51.9μL/min and could count microparticles with a high rate of 1700 particles/s [25].

Groisman et al. [26] proposed and designed a high throughput cytometer based on microfluidic systems. This high throughput has been achieved by increasing microchannel size to 110×300μm. Using a sheath flow, particles are adjusted in the center of the channel where the flow velocity is maximum and stable. This sheath flow confines the particle to a region with a diameter of 5-10μm. With these modifications and integration of a confocal microscope as fluorescence obtainer, they could increase throughput value up to 17000 particles/s with particle velocity of 8mm/s. In their assay, 1.9μm polystyrene beads were used with a volume concentration of 2.8×10^5 particle/μl which is close to RBC concentration in human blood sample. In other words, by increasing their setup size, one can be able to flow samples with undiluted volume concentrations.

1.2.2. Microcantilever based diagnostics

Microcantilever biosensors work based on mechanical deflection measurement and frequency variation detection to measure bioelements and cells properties such as internal pressure, applied force and mass flow rate. Study of functions of mechanical forces in cell biology is called mechanobiology. This investigation has great influence in various fundamental biological processes such as cell growth, differentiation and morphogenesis. To this end, parameters such as type and magnitude of cellular forces, function of forces in cell biology and cell mechanical properties have been studied under this category. In this regard, a great amount of research has been conducted on cell biochemical reaction for different force signals [27, 28, 29]. Although these studies have clarified cells biochemical feedbacks to external forces, the main shortcoming in such studies is the lack of precise manipulation with determined amount of force and measurable cell feedbacks. This issue has been addressed using microsystems in cell mechanobiology.

A) Deflection based microcantilevers

Microcantilever systems have covered the need for measuring applied forces to cells and detecting their tensile and contractile reactions. As an example, Galbraith et al. [30] measured traction forces of fibroblasts using an array of microcantilevers. Force responses

of a single bovine endothelial cell attached to a substrate was studied by Saif et al. [31]. They used a microcantilever which is made of single crystal silicon and a piezoactuator to move the microcantilever against the cell. Cantilever deflections were recorded using image analysis resulted due to interaction force between cell and microcantilever. Measuring applied tensile forces was the subject of another study done by Yang et al. [32]. They designed a microcantilever system with an integrated micro force sensor to measure the reaction of fibroblasts to tensile forces. Real-time extraction of contractile forces of cardiomyocyte cells has been subject of another investigation conducted by Park et al. [33]. In this study, they have designed and tested a hybrid polymeric microcantilever which is made of PDMS.

Cells or plants growth rate is bonded to internal force of cell (turgor pressure) and measuring this mechanobiological parameter has been challenging since its discovery. There are several studies in the past century that looked at this mechanobiological factor of cells. However, before advancements in MEMS, all studies are either imprecise or limited to certain species [34, 35, 36, 37]. The first micromanipulation of plant cells was done by Blewett et al. [38] in a study of turgor pressure of single tomato cell. They trapped a cell between a microprobe and a surface. By applying a known amount of force and monitoring the images of the deformed cell, they finally calculated turgor pressure inside cell by dividing force by deformed area of the cell (Figure 1-7).

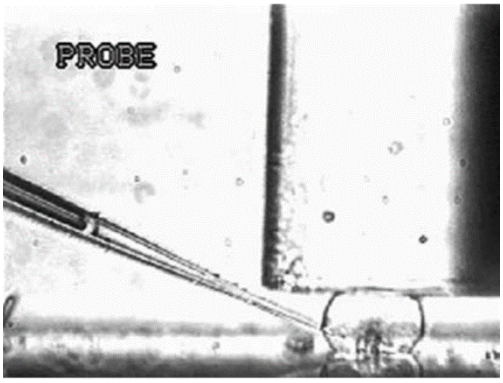


Figure 1-7. A 200μm probe was used to extract turgor pressure of a single tomato cell [39]

Invasive forces of Hyphae cell was studied and measured by Money et al. [40]. To this end, they used a microcantilever which was made out of a silicon with size of 10×1×0.1 mm.

Using this microcantilever, they measured a mean force of 12μN for Hyphae cell of 15-25μm size. The internal pressure of Pollen tube has been the subject of several studies. In one of the most recent one, Ghanbari et al. [41] have measured this mechanobiological parameter using a microcantilever within a microfluidics (Figure 1-8). Using this set up they measured the microcantilever deflection and consequently Pollen tube internal pressure. Using mechanical properties of the PDMS microcantilever (E=750 kPa, v=0.45) the stiffness was determined k=0.023 μN/μm. Thus, the amount of applied force for deflection of 67μm was shown around 1.53 μN. Dividing this force by Pollen tube diameter, the maximum internal pressure was found 0.19MPa.

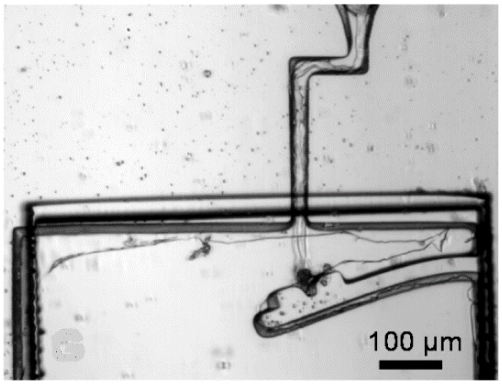


Figure 1-8. PDMS microcantilever system used for extraction of Pollen tube internal force and pressure [41]

B) Frequency based microcantilevers

Adsorbed mass on microcantilever directly affects its natural frequency which can be employed as mass detector. This frequency shift depends on position of the added biomolecule, mass and vibration mode under investigation. In order to design a frequency-based microcantilever, the dimension of particles under investigations is the defining parameter for microcantilever dimensions. In other words, the smaller particle measurement, the smaller micro/nanocantilever required. On the other hand, lesser the particle size/mass, the higher quality factor required. Regarding size, there are some methods to fabricate cantilevers in nano dimensions. However, nanofabrication techniques are highly irreproducible in dimensions and mechanical properties [5]. These are main barrier in front of nano-structures to be employed as real-time measurement systems such as microsystems. Regarding quality factor, the bioparticle size defines the required quality factor as precision

parameter. The quality factor should be in the order of 1,000-100,000 for micro/nanocantilevers which is difficult to reach due to the medium around the microcantilever.

To deliver biomolecules on a microcantilever, a liquid medium is usually employed. However, this medium will drastically decrease quality factor to the order of 1-10. Quality factor is defined as stored vibrational energy over dissipated energy in each cycle. In other words, liquid medium has high dissipating range. To overcome the low quality factor issues of microsystems soaked in fluid, some solutions have been proposed and tested such as using other vibration modes and using ex situ method. Exciting higher vibration modes was experienced by Braun et al. [42] which shows exciting of 16^{th} mode instead of 1^{st} one increases quality factor from 1 to 30. In another investigation, Tamayo et al. [43] increased quality factor in water medium using an active amplification method. They showed that quality factor of microcantilever in liquid medium which is around 1-10 can be increased by three orders up to 1000 through their Q-control method. A quality factor of 625 in liquid medium and detecting an antibody, STAR71, with concentration of 0.8 μg/mL were achieved. Although this technique is practical for quality factor improvement up to 1000, variation of local viscosity amounts due to temperature changes in fluid makes unstable vibrations for higher quality factor values.

Ex situ measurement is a method where measurement is done in air/vacuum after rinsing the microcantilever inside the bioelements solution. This method was employed by Ramos et al. [44] for DNA analysis. The main drawback of this method is the risk of contaminations after rinsing and degrading the measured parameters. Although applying this method is very risky to get valuable results, Craighead et al. [45] measured prostate specific antigen with sensitivity of 50 fg/ml. In this experiment, a microresonator made of silicon nitride using lithography technique was employed. After measuring the resonator natural frequency in vacuum, it has been functionalized with appropriate receptors to detect prostate antigens. In this step, microstructure is soaked in a diluted mixture of nanoparticles and washed and dried after 90 mins exposure to the solution. Finally, resonance frequency is measured by exciting the microstructure on an external piezoelectric element.

The only remaining solution up to now to address the fluid medium drawbacks is to replace liquid with air/vacuum. But, new question is how to deliver biomaterials without fluids. Roukes et al. [46] delivered bioparticles using electrospray injection from fluid to vacuum medium. Frequency monitoring shows real-time shifts when each particle hits the nanocantilever. In this experiment, the mass of 66kDa (1.09×10^{-10} ng) of BSA and 200kDa of b-amylase were measured. This technique works based on mass spectrometry which ionize particles and measures mass to charge ratio however, some molecules are not suitable to be ionized and may be damaged.

High quality factor in microcantilever systems without fluidic medium made them a promising tool to measure mass of any particle even in molecular orders. Moreover, these microstructures have been widely used in biological detection and experiments. Lee et al. [47] employed such microcantilever to detect the prostate-specific antigen through measuring frequency shift. Using such method, samples with concentration of 10 pg/ml were detected. Repeating this experiment for two microcantilevers with different dimensions, it was proved that detection of lower concentration of any cell/particle requires smaller microcantilevers. Craighead et al. [48] used this technique to detect single cell of E. Coli by measuring frequency changes of a microcantilever in vacuum condition instead of atmospheric pressure to increase the sensitivity of their microsystem to 1.1 Hz/fg (Figure 1-9).

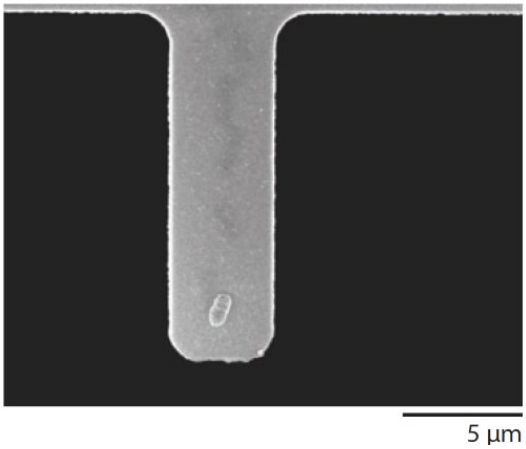


Figure 1-9. A scanning electron microscope view of a single E.coli cell adsorbed to a microcantilever resonator [48]

1.3. Integration of microfluidics and microcantilevers

Although microcantilevers have shown great promise in mechanobiological study of cells, delivering cells mixture to the microcantilever needs a fluidic medium which limits the sensitivity of microsystem. This low sensitivity is due to low quality factor of moving microstructures in fluidic mediums since damping there is much higher than air or vacuum. Moreover, evaporation of fluid that happens in fluid around microcantilever in ex-situ techniques, affects the repeatability of the results. On the other hand, microfluidics based diagnostics need complex and expensive measurement systems such as optical devices.

To overcome these drawbacks, a solution of combining microfluidics into microcantilevers in order to benefit from both techniques and minimize the disadvantages was proposed which is suspended microchannel resonator (SMR) method [49]. This microsystem has been designed and fabricated in silicon chip which makes it very compact, with high sensitivity and repeatability. The only drawback with this suspended microsystem is its high stiff silicon microcantilever which cannot be deflected by cells without use of an external electrostatic excitation. Using this external excitation, frequency change of suspended microchannel is monitored which results in extraction of biophysical properties of grabbed or flown cells and bioparticles. This suspended microfluidics measures the mass of cells or molecules in two different methods through monitoring vibration frequency of microcantilever. First, when the microchannel walls are functionalized with appropriate receptors, particles are trapped within the microchannel which consequently varies the vibration frequency of the microcantilever. Second, when particles are just passing through the microchannel and are not trapped there, the frequency of microcantilever shifts when the position of the particle varies. Figure 1-10 shows two main working ways of SMR (adsorbing or flowing particles) which can be used for biophysical cell analysis such as weighing, counting and etc.

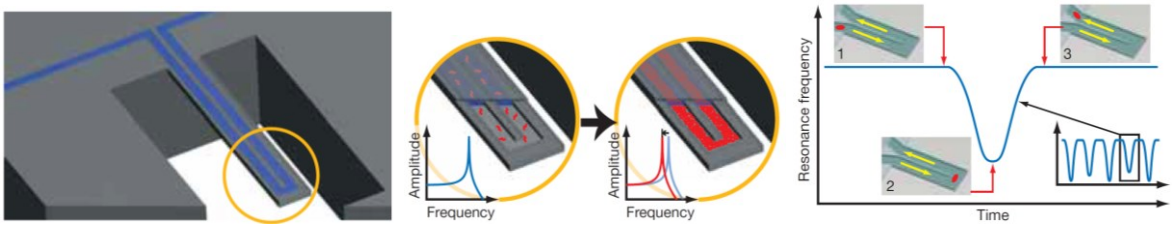


Figure 1-10. Suspended microchannel resonator (SMR) made out of Silicon [49]

Burg et al. [49] showed that the SMR is capable of measuring particles of 1 fg (10^{-15}g). This sensitivity is owed to a high quality factor of 15,000 which is not reachable by microcantilevers surrounded by fluid. The only shortcomings of this SMR system are its low throughput of 10-100pl/s and employment of high stiff silicon in microfabrication which requires external exciter for frequency measurements. To achieve high resolution and sensitivity of 1fg, the flow rate inside microchannel was reduced in a way that only 1-10 particles or cells per second pass through the microchannel. On the other hand, this low throughput enabled researchers to measure mass of individual cells which resulted in measuring E. Coli and Bacillus bacteria cell masses with high accuracy in comparison with other investigations (Figure 1-11).

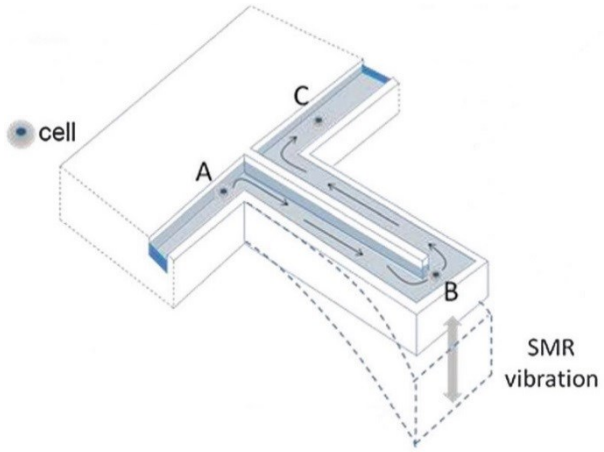


Figure 1-11. Schematic view of suspended microchannel resonator [5]

The technique of suspended microchannel has been employed for other applications such as particle volume and density measurement. Bryan et al. [50] have designed a dual SMR system to measure physical properties of cells in which, two different fluids with different densities are used in each microchannel. While a cell/particle passes through each of these microchannels, its buoyant mass is measured differently based on the fluids' density. After these two measurements, the physical properties of cells such as mass, volume and density are derived. Moreover, the other embedded parts have been modified for better resolution and packaging. For example, Lee et al. [51] replaced the laser deflection measurement with

a piezoresistive bridge. This will package all the SMR microsystem as an electrical system which offers a comparable resolution to optical displacement method.

To get rid of adding external excitation to SMR, the technique of making this microstructure out of a polymer such as PDMS has been introduced and tested. SadAbadi et al. [52] designed and tested this new suspended microfluidics which is less stiff than the silicon one so that cells and bioparticles can deflect it with their physical properties such as mass. Using this method, they have designed a suspended microchannel with size of 1.2×4 mm in which a 15×200 µm microchannel is buried (**Error! Reference source not found.**). In order to ncrease the SPMF sensitivity, they have optimized place of the embedded microchannel plane above microcantilever neutral axis as well as addition of gold nanoparticles to the suspended microchannel.

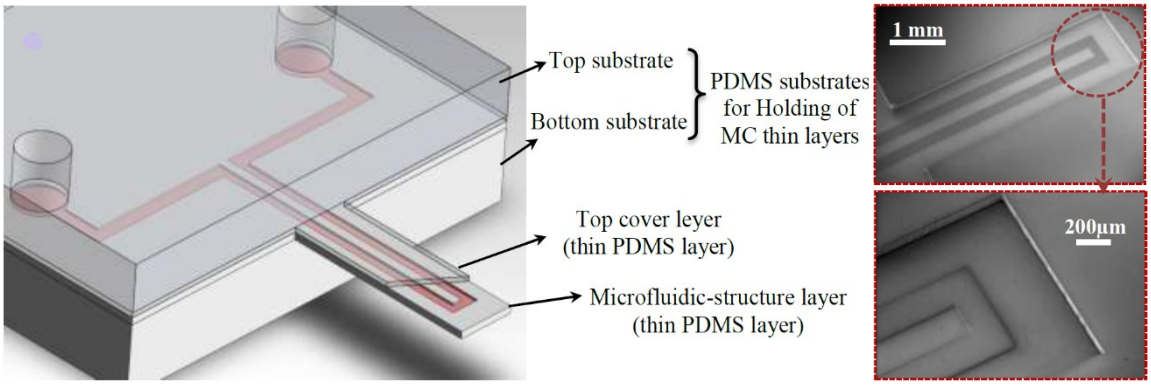


Figure 1-12. Schematic and mold view of suspended PDMS microchannel [52]

This microfluidic system has been tested with injection of anti-bovine growth hormone inside the microchannel which showed a tip deflection of 20µm with a concentration of 80ng/ml. Based on their results, the minimum detectable concentration of the hormone is 10ng/ml.

1.4. Thesis motivation

Measuring biophysical and mechanobiological properties of cells may have been accomplished using microcantilever systems, as it is discussed in Section 1. These microsystems have shown valuable results though there are still some issues to be addressed. The main drawbacks of microcantilevers are the method of delivering bioparticles to microstructure area, its sensitivity and repeatability. To overcome these issues, some

methods have been proposed in the literature at which the one that can address all of these issues is to employ suspended microchannel inside a microcantilever for delivering sample under study. In other words, removing fluidic medium around microcantilever increases the quality factor, and consequently its sensitivity, to an extent which is enough to detect even single cell's mass. Repeatability issue will be resolved as well while there is no fluidic medium around microcantilever to deliver cell sample. In this technique, every analysis is done inside a closed loop microchannel which controls the sample flow and prevents from sample evaporation.

As it is mentioned earlier in Section 1.3, the embedded microchannel inside SMR has drastically increased quality factor in microcantilever systems. However, there are few issues in each study on SMR that should be considered in further investigations. Flow rate in SMR microstructures is not even comparable with its optical and electrical competitors. Moreover, optical micro cytometers have higher throughput and are more sensitive in comparison with their electrical competitors. The high sensitivity in optical cytometers is achieved by the employment of fluorescence labeling as well as high precision optical instruments which are bigger and more expensive in comparison with microchannels. On the other hand, SMR systems have very low throughput but, very high potent in micromanufacturing and final size. In other words, as it is shown in earlier studies, by increasing the suspended microchannel size, its flow rate will be drastically increased though, sensitivity should be compromised with the bioparticles size.

Another solution for resolving suspended microfluidics issues is to look differently at this innovative microsystem. The concept of cell mechanobiological study through measuring physical parameter changes in microfluidic channels is proposed in this study. So far, frequency variation is the transduction principle of SMR system. However, these microsystems have another aspect which can be used for bioparticle analysis and detection too. This aspect is the momentum change in fluid flow due to change in flow direction or size variation of the microfluidic channel which applies a force to the suspended microstructure. Monitoring this force has been one of the practical methods in commercial flow meters. Adding microparticles to this microfluidic flow applies another force to the fixtures due to variation in the microchannel area. Therefore, a 3D suspended polymeric

microfluidics ($SPMF^3$) is introduced in this study for more sensitive diagnostics compared with the fellow microsystems.

In order to intensify the sensitivity of the proposed suspended microfluidics, the microchannel plane is rotated by 90 degrees to be perpendicular to the microcantilever plane. Besides, the applied force is augmented by modifying microchannel dimensions such as addition of a micro-nozzle between microchannels. Furthermore, changing the material of microcantilever from highly stiff silicon to PDMS will also increase the amount of sensitivity. Monitoring the microcantilever deflections provides a new and innovative method to detect and study biophysical properties of bioelements. Figure 1-13 shows a 3D suspended microfluidics that employs flow forces as transduction principle.

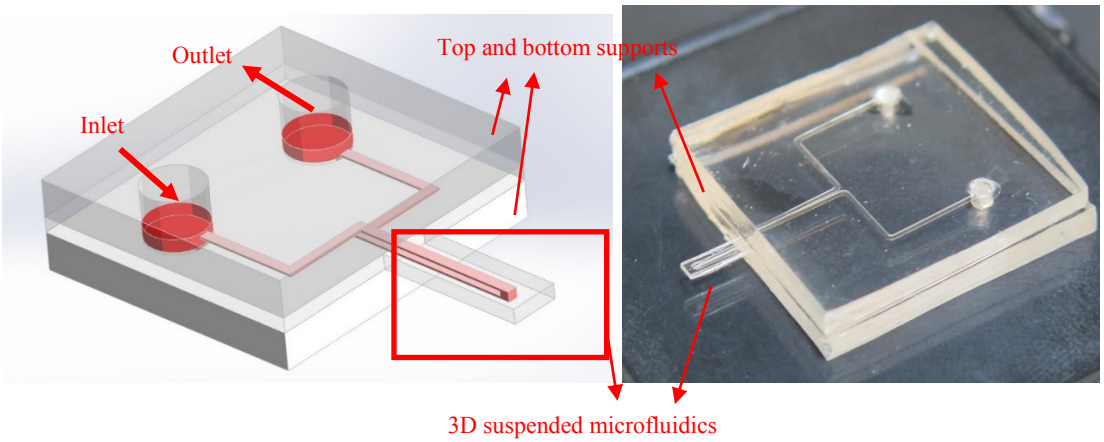


Figure 1-13. 3D suspended polymeric microfluidics for sensitive diagnosis

1.5. Thesis objective and scope

The main objective of the present thesis is to design and develop a suspended polymeric microfluidics for sensitive biodiagnostic applications. The proposed microfluidics detects and studies bioelemenets and extracts their biophysical properties such as size, weight and flexibility. Moreover, in colloidal fluids or solutions with multiple compartments, any variation in the substance concentration and their effects on the fluid properties such as viscosity and density can be detected. This detectability comes from flow forces applied on the microcantilever inside which the 3D microchannel is embedded. In other words, this innovative biodiagnostic platform employs microfluidic flow forces as transduction principle instead of frequency or stress variations used in the fellow microsystems.

In order to achieve the main objective of this thesis, the following sub-objectives have to be reached.

1- Design and development of a suspended microfluidics without external excitation for creating deflections which is sensitive to microparticles and bioelements flow
2- Development of a fabrication process for the suspended microfluidics
3- Optimizing different design and fabrication parameters such as microchannel size, offset and nozzle dimensions for higher sensitivity
4- Measuring steady and pulsatile flow rates
5- Detecting variations in fluid properties such as viscosity and density
6- Detecting and studying microparticles flow using the suspended microfluidics

1.6. Contributions of author

In this study, the 3D suspended microfluidics was designed, simulated and optimized for fabrication. Then, the $SPMF^3$ was fabricated using soft lithography technique and manufacturing issues were resolved in order to get the required microfluidics quality for the upcoming experiments. These results as well as experimental validation of design was submitted to the Lab on a Chip journal as follows. Another application of the $SPMF^3$ is to detect the variations in fluid properties. Measuring fluid properties such as viscosity and density is a technique to specify fluid status specifically in some biomedical applications. In this experiment, different concentrations of salt in DI water and milk with different fat content was tested using $SPMF^3$ and the results were verified with theoretical model. The outcome of this experiment was the interpretation of kinematic viscosity and the importance of employing that for specifying certain types of fluids when being diagnosed which was submitted to the journal of Biosensors and Bioelectronics.

Finally detecting and studying biophysical properties of bioelements were done by the $SPMF^3$. The polystyrene microbeads and air bubble were used to mimic hard and flexible bioparticles. In this experiment, it was shown that microparticles can be detected using the $SPMF^3$ without external exciter. Moreover, biophysical properties of the bioelements such as size and flexibility can be extracted and studied. These results were submitted to the journal of IOP Micromechanics and Microengineering.

There are three other papers that are almost ready to be submitted to the following journals during or after thesis defense. As is was mentioned earlier, the fabrication process of the $SPMF^3$ is a novel process that is going to be submitted in journal of Microsystem Technologies with the title of "Microfluidic Air Vent Assisted Bonding for Thin Layered Polymeric Devices". Furthermore, the FEA modeling work which is explained in part in chapter two is also ready to be submitted to a scientific journal with the title of "Coupled Modeling of Fluid Structure Interaction of Suspended Polymeric Microfluidics". The third paper in pipeline is related to experimental design of the $SPMF^3$ based on the results that are partly presented as the Appendix A of the thesis. This paper is titled "Optical Compatible Measurement Arrangement of the $SPMF^3$". The title and summary of three submitted journal papers are as follows.

1- Lab on a Chip journal (Chapter 3)

Flow Force Augmented 3D Suspended Polymeric Microfluidic ($SPMF^3$) Platform for Sensitive Diagnosis

In order to improve repeatability and sensitivity of biosensing microsystems, an innovative 3D suspended microchannel has been proposed using which microparticles can be conveyed through a microchannel inside the microcantilever to the detection area. This innovative microchannel design addresses the low sensitivity issue by increasing it up to 5 times more sensitive than the reported similar microsystems. Moreover, fabricating this microsystem out of Polydimethylsiloxane (PDMS) will eliminate external exciter dependency in many detection applications such as biodiagnostics. Detection of bioelements using microcantilevers and microfluidics has been of great interest in the biodiagnostics field. Microcantilevers are the most used systems in biodetection due to their implementation simplicity. These microsystems have been used for a wide variety of applications ranging from cellular to molecular diagnosis. In this study, the designed microsystem has been analyzed theoretically and simulated. Moreover, the microsystem has been fabricated and employed in several experiments, the results of which have been compared with simulation results. Finally, its innovative fabrication process and issues are reported and discussed.

2- Journal of Biosensors and Bioelectronics (Chapter 4)

3D Suspended Polymeric Microfluidics ($SPMF^3$) with Flow Orthogonal to Bending (FOB) for Analysis of Newtonian and non-Newtonian Fluids through Kinematic Viscosity

Measuring variations of dynamic viscosity during fluid processes is bonded to a knowledge of different concentrations of fluid compartments and their densities in a solution. To avoid dealing with the fluid density, detection of kinematic viscosity is proposed in this study. Kinematic viscosity, also called as momentum diffusivity, considers both changes in fluid intermolecular forces and molecular inertia which defines dynamic viscosity and fluid density, respectively. In this paper, a 3D suspended polymeric microfluidic systems ($SPMF^3$) was employed to detect changes in fluid parameters such as dynamic viscosity and density. Using this innovative design and according to theoretical and experimental results, it is shown that in fluids, variations of the fluid density and dynamic viscosity are not easily comprehensible due to their interconnectivity. Since any change in fluid parameter will affect both density and dynamic viscosity, measuring both of them is necessary to identify the fluid. Finally, changes in fluid properties were detected using simulation and experiments. The experimental results of salt-DI Water solution and milk with different fat concentration as a colloidal fluid show that kinematic viscosity is a unique parameter that can identify the status fluids.

3- Journal of IOP Micromechanics and Microengineering (Chapter 5)

Rigid and Flexible Microparticles Detection Using A 3D Suspended Polymeric Microfluidics

In this paper, different methods for cell and microparticles detection was compared and the innovative 3D suspended polymeric microfluidics ($SPMF^3$) for microparticles detection was introduced and tested. Microsystems have gathered great interests and shown valuable results in the study of mechanobiology and biophysical analysis of cells due to their properties such as: microsystems match cell dimensions, provide growth microenvironment such as in-vivo environment, facilitate parallel analysis that can be done on cells through integration of other sensors or chemicals to the microsystem, low cost, low amount of sample use, etc. The $SPMF^3$ is less complex and less expensive compared with the other optical,

microfluidics and microcantilever based techniques formerly employed for microparticles and cell detection. According to the experimental results, the SPMF^3 is sensitive to the particles and bubbles when the pass through the nozzle. Counting number of peaks in the microcantilever deflection measurement graph, one can study and count the size and number of passed particles.

1.7. Organization of the thesis in manuscript based format

This thesis is submitted in a manuscript based format in which all the chapters excluding first, second and final chapters, are duplicated from the manuscripts were submitted and are under reviewing process for publication in scientific journals.

In the first chapter, an introduction on sensitive biodiagnostics as well as a brief literature review on a variety of current methods for detection and studying bioelements is presented. Later in each chapter, a detailed literature review regarding each topic is gathered. In Chapter 2, the details of suspended microfluidics design and FEA modeling process is explained. The following chapters also present some FEA results of this thesis depending on the chapter subject. Chapters 3, 4 and 5 are duplicated from the three submitted journal articles. Chapter 6 contains the concluding remarks and recommendations for future works. Finally, there is an appendix of the thesis including experimental setup design and implementation details which could not be mentioned in the submitted articles.

To comply with the Concordia thesis regulations, figures, tables and equations numbers may have been modified from the original submitted article. As such, the reference lists of all papers are combined and presented at the end of the thesis.

Chapter 2

Finite Element Modeling and Simulation

As it is mentioned in the first chapter, this thesis is submitted in the manuscript based format in which each chapter excluding introduction, conclusions and current chapter are submitted to scientific journals. However, in order to reach the results which are presented in the following chapters 3-5, some initial steps were performed which are documented in this chapter for future results reproduction which will be published in a separate journal. Here, the early processes in design and simulation of the suspended microfluidics are presented and explained in details In order to verify the concept of high compliance 3D suspended polymeric microfluidics ($SPMF^3$) for biophysical study of cells, the first step is finite element analysis and modeling of a sample flow with microparticles inside a microfluidic system. Then, a 3D high compliance suspended microfluidics is designed and modeled based on these results. The initial step in 3D microfluidics will be design and modeling of microfluidic-microcantilever interactions and its sensitivity to flow forces and bioelements flow.

2.1. Design

In this section, first microparticles flow inside a straight microchannel and fluid parameters will be analyzed and measured. Then, a suspended microfluidics design will be studied in order to design a highly sensitive 3D suspended microfluidics. The 2D modeling analyses are done using COMSOL for its simplicity and the complicated 3D fluid structure interaction (FSI) modeling of the 3D suspended microfluidics is carried out in ANSYS.

2.1.1. Physical modeling of a microchannel with microparticles flow

A) Straight microchannel:

To prove the concept of flowing cells and microparticles inside a microfluidic system and examine their effects on flow parameters inside microchannel such as local flow pressure and speed, a finite element analysis (FEA) was performed. This FEA modeling has been done in COMSOL environment using design parameters which are presented in Table 2-1. In this analysis, particles are passing through a microfluidic channel of 40 µm width and flow pressure at side wall of channel is measured. Besides, particles flow will influence on flow velocity profile and streamline deformation which is shown in Figure 2-1. Based on the results, flow static pressure which is measured on the channel wall shows a drop as the particles pass with fluid flow (Figure 2-2).

Table 2-1.Parameters used for COMSOL modeling

Channel width (µm)	Channel length (µm)	Particle size (µm)	fluid	Velocity
40	200	20, 14	water	10 µm/s

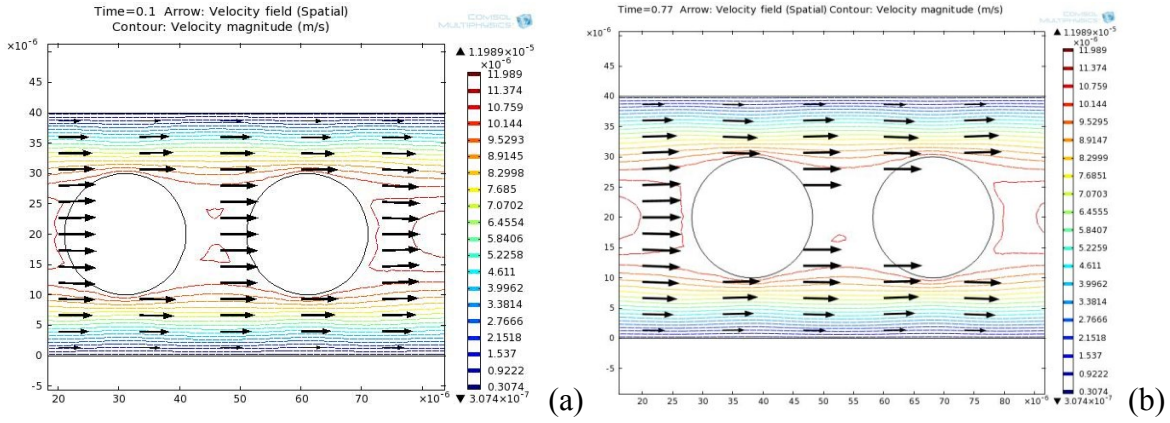


Figure 2-1. The contour of velocity streamline is deformed when particles moving from first point (a) to the second point (b)

As it is shown in Figure 2-2a, flow pressure drops by 0.45 Pa as a result of 20µm particle flow. Modifying flow parameters or particle features will affect abovementioned results. For example, injecting different microparticles with different sizes inside the fluid flow, provide different pressure and velocity changes in final simulation results. Figure 2-2b shows that

smaller particle flow (with 14µm diameter) in the same channel results in smaller pressure change on the measurement point. As another example, increasing flow speed or sample flow rate will also affect and increase the amount of pressure peak.

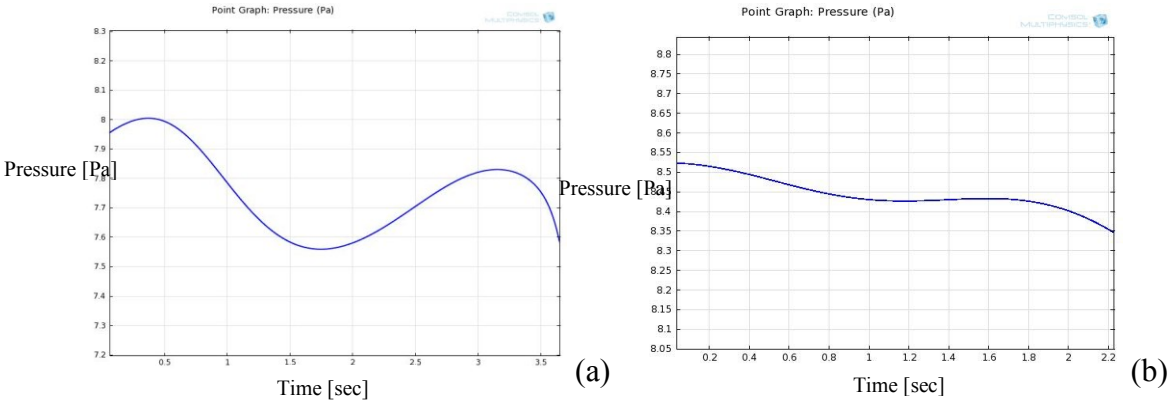


Figure 2-2. Pressure changes as particles flow inside the microchannel, a) 20µm particles, b) 14µm particles

B) U-shape microchannel:

Having a flow inside a U-shape channel or applying any bend in straight flow direction imposes a force to the structure by which the channel is held. This fluid structure interaction force which is called flow momentum force, is calculated based on flow parameters. The technique of changing flow direction to detect applied force on the structure has been used in some mechanical sensors such as flow meters which work exactly based on momentum change. Similarly, if we conduct a fluid flow with particles inside a curved microchannel, flow momentum change will apply a force on the microstructure. Moreover, particles create some pressure/velocity change pulses inside the microchannel as it is discussed and shown in Figure 2-2. These pressure pulses will create some force pulses and consequently results in vibrational deflection of suspended microsystem. Monitoring deflections of microcantilever derived by momentum force pulses will result in detection, analysis and counting of cells and bioelements.

To make this technique more clear, two finite element simulations have been done. First one shows the effects of fluid flow momentum change on a U-shaped microchannel. Second one is simulated with a flow while the flow velocity is oscillating. To this end, a 2D model of the microchannel was simulated and amount of applied forces to the channel walls were

extracted. Figure 2-3 shows a schematic view of the suspended microfluidics used for FEA modeling and study of flow inside U-shape microchannel.

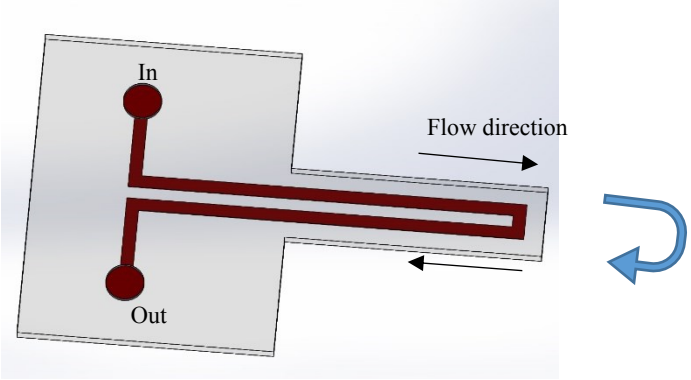


Figure 2-3. Schematic view of a 2D suspended microchannel parallel to the cantilever neutral plane

This FEA modeling has been done in COMSOL environment using design parameters which are shown in Table 2-2. As it is shown in Figure 2-4, this modification in flow direction applies a force on the microchannel which results in channel deflection if it is not stiff or fixed.

Table 2-2. Parameters of suspended microstructure used for COMSOL simulation

Channel size (µm)	Cantilever size (µm)	Fluid	Velocity (µm/s)	PDMS Young modulus (E, kPa)
200×15	2100×1200×165	water	30	700

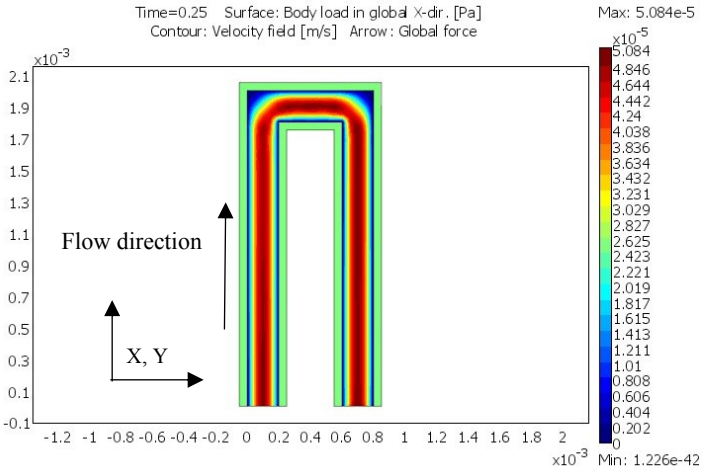
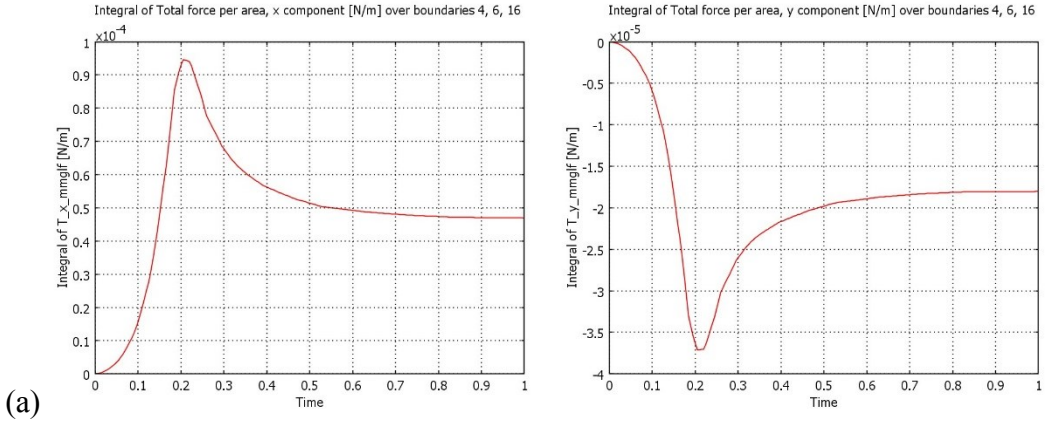


Figure 2-4. U-shaped channel modeling under flow forces

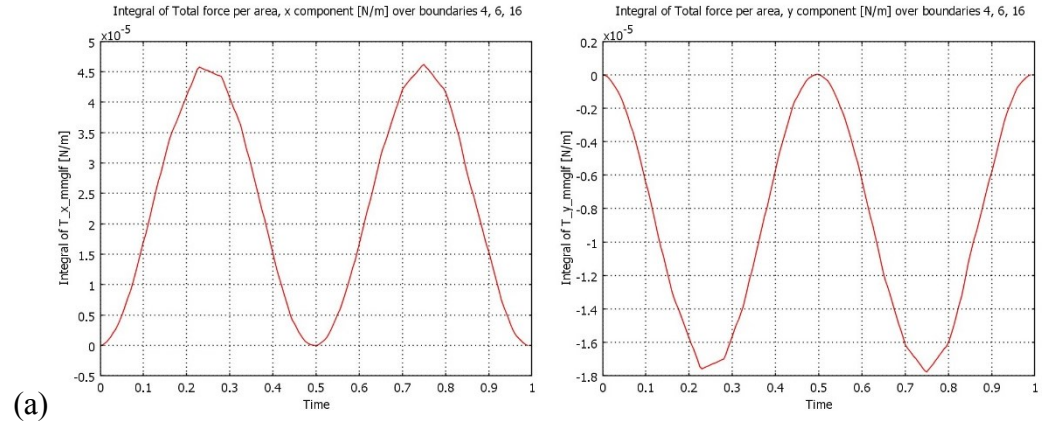
Using this FEA modeling, the amount of applied force to the channel walls have been extracted and shown in Figure 2-5. In this simulation, flow speed is gradually increased to 50 µm/s peak and then it settles to the predefined value of 30 µm/s. thus, following figure shows a peak in amount of applied force in X and Y directions and then settles to the steady state value.



(a) (b)

Figure 2-5. Amount of applied force to the microchannel wall, X-direction (a), Y-direction (b)

As it is apparent from the problem physics, variation in the flow parameters such as speed and flow rate will directly affect the momentum force and consequently the channel deflection. In the second simulation, a flow with impulsive fluid velocity is conducted inside the channel. These velocity pulses mimic the particles flow inside the U-shaped microchannel. Since it was shown and approved in the last section that particle flow will affect flow velocity and pressure, this impulsive velocity flow assumption seems reasonable. This simulation results in the channel force pulses with impulsive velocity flow which is shown in Figure 2-6.



(a) (b)

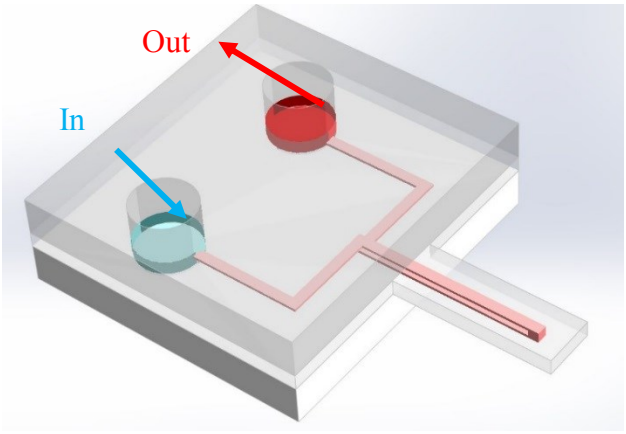
Figure 2-6. Applied force on the 2D suspended microchannel under impulsive velocity input, X-direction (a), Y-direction (b)

Based on these results, changing the fluid flow parameters such as flow velocity and it's frequency to imitate the real situation of different particle sizes and numbers will show that if velocity pulse amplitude increases, applied force to the channel (deflection amplitude) will be amplified.

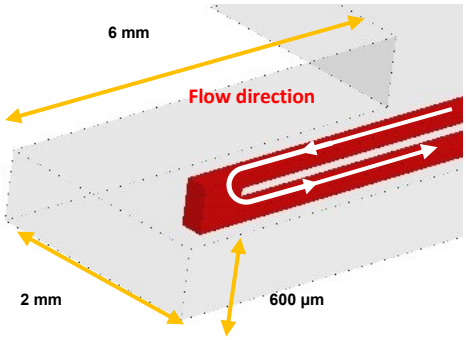
C) 3D Suspended Microfluidics Design and Identification

A tested microsystem for detection and study of microparticles is Suspended Microchannel Resonator (SMR), Figure 2-3, which works based on frequency analysis of the suspended microsystem while a particles passing through microchannel. However, an external exciter is used to move microsystem since the amount of applied force is very low to bend a stiff SMR which is made out of silicon. Sadabadi et al. [52] tried to resolve this issue by replacing silicon with PDMS. This technique has been investigated and called suspended polymeric microfluidics (SPMF). Using this method, they have designed a suspended microchannel with size of 1.2×4 mm in which a 15×200μm microchannel is embedded.

The current design of suspended microfluidics is not able to detect the abovementioned flow forces (Figure 2-3). Since, the silicon SMR and suspended PDMS microsystem have a microfluidic channel in plane of microcantilevers, the flow and microparticles forces are applied perpendicular to microcantilever deflection direction. Thus, the microchannel configuration has to be modified in a way that sensitivity on suspended microsystem allows this force to deflect it. To this end, a new concept of 3D suspended polymeric microfluidics ($SPMF^3$) has been introduced in this study (Figure 2-7). Changing the microchannel plane to be orthogonal to the microcantilever neutral plane, will drastically decrease moment of inertia and increases sensitivity of designed microsystem. Furthermore, changing the material of microchannel from silicon to a flexible material such as PDMS will also increase the amount of deflection or sensitivity to small loads. Monitoring these deflections will provide an innovative method to detect and study biophysical properties of cells based on flow forces. Some other explanations regarding the $SPMF^3$ detail design are presented and discussed in chapter three which is submitted to a scientific journal.



(a)



(b)

Figure 2-7. a) Designed 3D suspended microfluidics b) detailed view of the microcantilever

In order to understand and proof the concept, a detailed FEA modeling has been done using ANSYS to extract fluid and structural behavior of the proposed microchannel under simulated load conditions. In this simulation, first a fluid with different properties such as density and viscosity was injected and fluid dynamics parameters at the microchannel were measured and discussed as follows. Table 2-3 shows the dimensions of the microcantilever with a 3D embedded microchannel which are employed in this analysis.

Table 2-3. 3D suspended microfluidics design parameters

Cantilever size, L, W, T (µm)	Microchannel size, W, T (µm)	Speed (mm/s)	Fluid
6000×2000×600	200×100	30	Water

In this simulation results, effects of changes in fluid density and viscosity are studied in the vicinity of the nozzle area located between two microchannel layers of designed microsystem. Figure 2-8 shows the microchannel with desired study points in fluid dynamics and the simulation results are gathered in Table 2-4. These results are discussed from another point of view in chapter three.

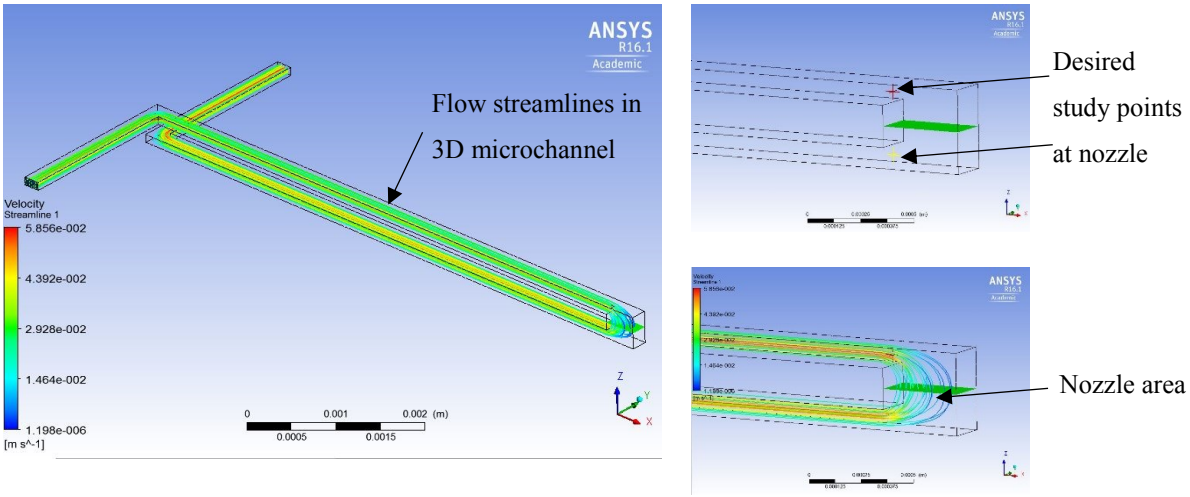


Figure 2-8. The 3D microfluidics simulation using ANSYS-CFX

Table 2-4. The fluid structure interaction simulation results with constant input velocity

Density (kg/ m^3)	Viscosity (Pa s)	Inlet velocity (m/s)	Velocity at Nozzle (m/s)	Delta Velocity at Nozzle (m/s)	Delta Pressure at Nozzle (Pa)	Pressure Nozzle inlet (Pa)	Reynolds	Velocity at Nozzle inlet (m/s)	Deflection (μm)
997	0.00089	0.03	0.007124	0.002762	5.70361	369.381	2.12307	0.052636	8.0794
997	0.00097	0.03	0.006934	0.002566	6.04443	394.218	1.89594	0.051249	8.8091
997	0.00103	0.03	0.006791	0.002453	6.28445	411.727	1.74878	0.050148	9.3587
997	0.00111	0.03	0.006802	0.002373	6.83099	444.863	1.62522	0.049888	10.082
997	0.00119	0.03	0.0067	0.002282	7.22531	471.292	1.4933	0.048994	10.813
1100	0.00089	0.03	0.007129	0.002768	5.70761	369.589	2.34413	0.052687	7.3172
1200	0.00089	0.03	0.007132	0.002767	5.71149	369.661	2.55825	0.05269	6.7127
1300	0.00089	0.03	0.007135	0.002767	5.71561	369.732	2.77256	0.052694	6.1964
1400	0.00089	0.03	0.007138	0.002766	5.71991	369.802	2.98706	0.052698	5.754
1500	0.00089	0.03	0.007141	0.002766	5.72424	369.872	3.20174	0.052702	5.3704

As it is shown in the results, velocity drops as viscosity increases however, it does not change with density change. This will consequently increase pressure at that point which ends in more cantilever deflection. On the other hand, when density increase, effective mass of

cantilever is higher which results in cantilever bend in reverse direction which reduces total cantilever deflection. This simulation has been done with constant input velocity. In the next run, the constant flow rate has been analyzed since most of experimental devices work based on constant flow rate and the results are brought in Table 2-5.

Table 2-5. The fluid structure interaction simulation results with constant flow rate

Density (kg/ m^3)	Viscosity (Pa s)	Mass flow rate (g/s)	Velocity at Nozzle (m/s)	Delta Velocity at Nozzle (m/s)	Delta Pressure at Nozzle (Pa)	Pressure Nozzle inlet (Pa)	Reynolds	Velocity at Nozzle inlet (m/s)	Deflection (μm)
997	0.00089	0.000417	0.005004	0.001362	3.9966	259.897	1.49119	0.03708	9.30
997	0.00097	0.000417	0.004874	0.001332	4.25006	276.974	1.33272	0.035964	9.91
997	0.00103	0.000417	0.004713	0.001294	4.46362	291.184	1.23936	0.035408	10.4
997	0.00111	0.000417	0.004642	0.001206	4.92072	319.608	1.18079	0.03443	11.4
997	0.00119	0.000417	0.004547	0.001136	5.28104	343.012	1.10262	0.033464	12.3
1100	0.00089	0.000417	0.004433	0.001249	3.62131	235.442	1.49059	0.032597	8.43
1200	0.00089	0.000417	0.004157	0.001138	3.32098	215.917	1.49114	0.030805	7.73
1300	0.00089	0.000417	0.003837	0.001048	3.06479	199.266	1.49085	0.028426	7.13
1400	0.00089	0.000417	0.003564	0.000969	2.84665	185.101	1.49129	0.026404	6.62
1500	0.00089	0.000417	0.003324	0.000905	2.6553	172.674	1.49036	0.024625	6.18

According to the results, increasing fluid viscosity ends in velocity drop and pressure increase at the nozzle between two microchannel layers. Finally, similar to the previous simulation results, microcantilever deflections increase when fluid viscosity goes up and the deflections decrease when density increases.

The aforementioned results prove that this new concept of 3D suspended microfluidics is sensitive to particles or beads passing through the channel, pulsating flow and variation in fluid properties such as density and dynamic viscosity. Using this concept one can specify different kinds of particles and fluids passing through the microsystem.

2.2. Simulation

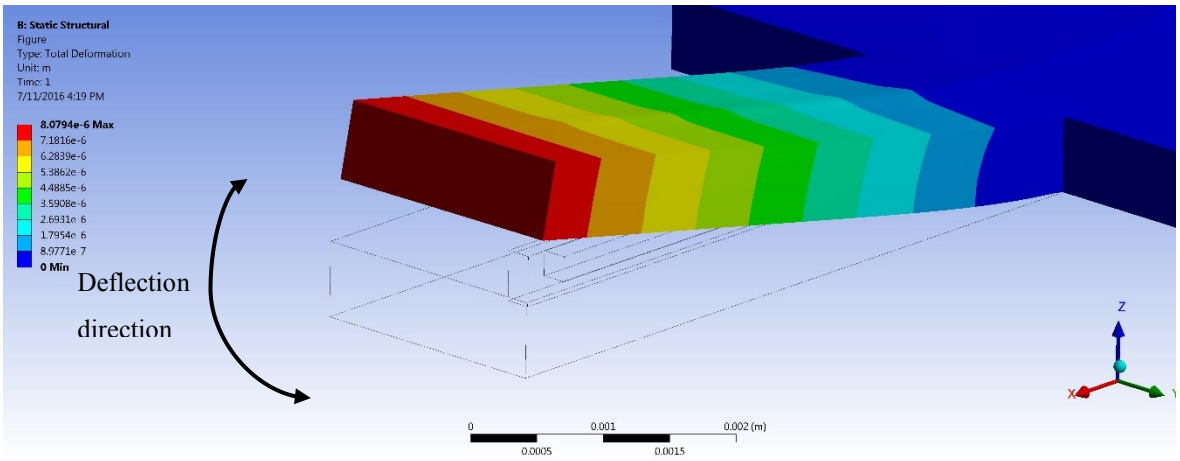
2.2.1. Detail design and optimization

After finalizing the concept, details of the proposed microsystem have to be determined for fabrication and experiments. In order to fabricate a 3D microchannel embedded inside a microcantilever, it is designed to divide it into three layers; two microchannels and one nozzle (aperture) by which these channel layers are connected after bonding and flow passes through whole microfluidics. In other words, these three elements can be optimized to have better sensitivity results with respect to the bioelements and cells under study. To this end, another finite element analysis with rough dimensions has been done as the first iteration. The target in this step is to see how the concept works and then, a sensitivity simulation is done to obtain the acceptable dimensions. Table 2-6 summarizes a few sets of dimensions that have been used as preliminary simulation and final acceptable dimensions.

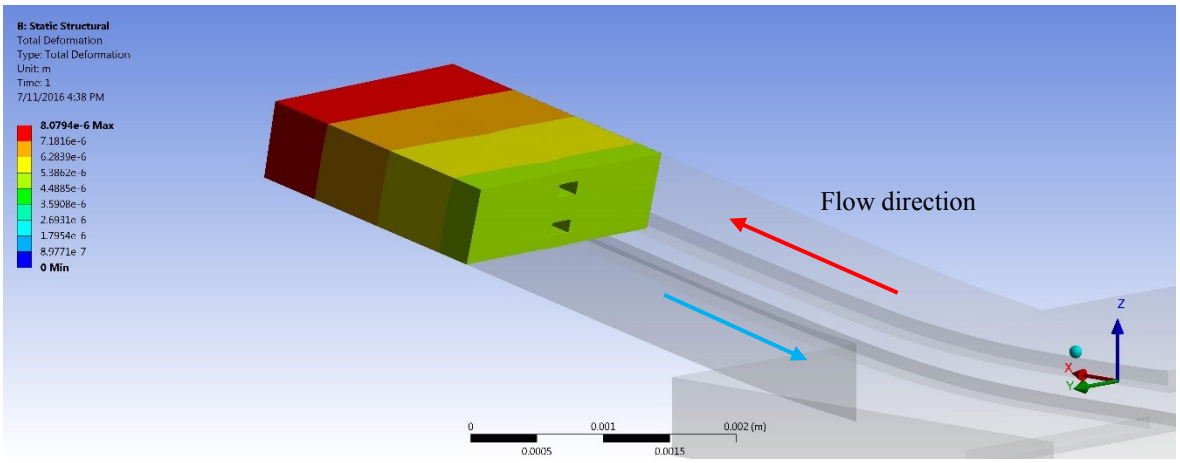
Table 2-6. Parameters used for suspended microfluidics simulation and resulting deflections

Design iteration	Cantilever size, L, W, T (μm)	Microchannel size, W, T (μm)	Nozzle size L, W (μm)	Speed (mm/s)	Deflection (μm)
1	6000×2000×165	200×15	200×15	10	0.809
2	6000×2000×300	200×50	200×200	30	3.196
3	6000×2000×600	200×100	200×400	30	8.079

Initially, to simulate and verify how the concept works, a microchannel with size of 200×15 μm has been embedded inside a microcantilever with size of 6000×2000×165 μm. Using FEA modeling, the maximum deflection of microcantilever under flow forces is extracted. The next step is to increase the sensitivity of microsystem to the applied loads. Here, the microchannel and microcantilever dimensions have been modified so that the amount of microcantilever deflection will be easily detectable at similar working conditions. At the same time the design intention is towards keeping the channel dimensions small enough so that the microsystem is sensitive to microparticles and cells which are going to be tested later. After some iterations, the simulation outcome for size of microchannel inside which microparticles with size of 70 μm or less can easily move, is 200×100μm and the microcantilever size will be 6000×2000×600 μm. Figure 2-9 shows the FEA results of this design process.



(a)



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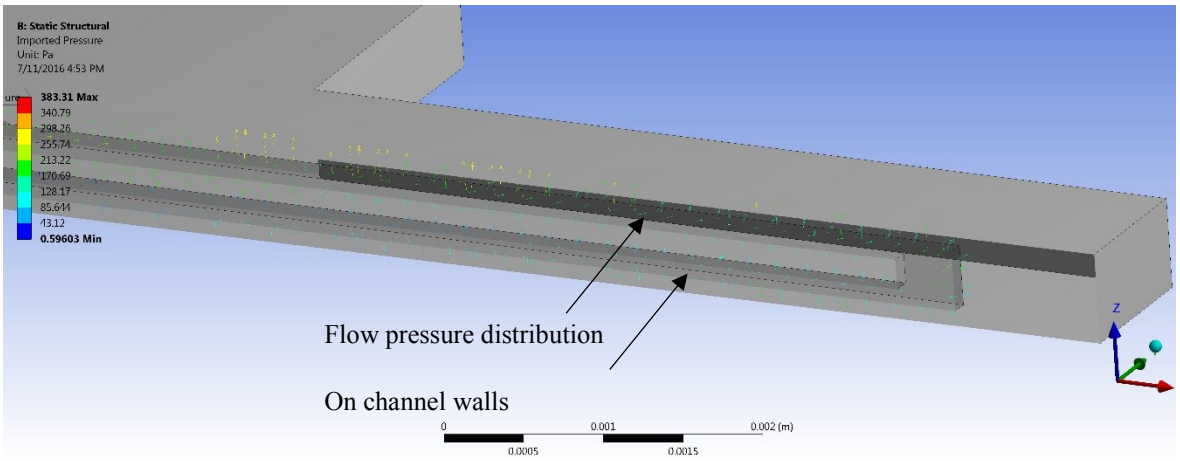
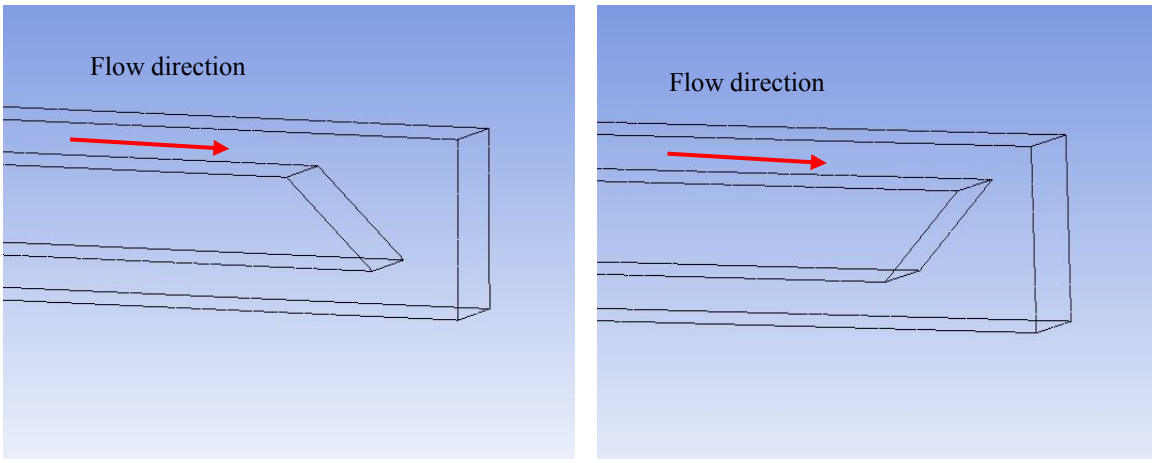


(c)

Figure 2-9. (a) Microcantilever deflection under steady flow, (b) section view of the microcantilever with the embedded microchannels, (c) applied pressure to the microchannel walls

Then, the optimization of nozzle size to improve microsystem sensitivity was considered and different nozzle shapes listed in the following Table 2-7 have been modeled. In order to simplify the nozzle shape optimization, two shapes of apertures have been considered shown in the following Figure 2-10.



(a) Nozzle (b) Diffuser

Figure 2-10. Two types of apertures simulated between microchannel layers, a) Nozzle, b) Diffuser

Table 2-7. Micro-nozzle parameters in simulation of the SPMF[3] with different apertures and the resulting deflections

Design iteration	Nozzle size (inlet), L, W (μm)	Nozzle size (outlet), L, W (μm)	Mass flow rate (g/s)	Deflection (μm)
1- Nozzle	400×200	200×200	0.000416	15.4
2- Diffuser	200×200	200×400	0.000416	26.1

Using the aforementioned data, a detailed simulation has been done to investigate the effect of each aperture shape on the microsystem deflection. First, a nozzle with sizes of 400×200 and 200×200 was modeled and the detail results are summarized in Table 2-8. As it is shown in the results, Table 2-7, microcantilever deflection using a nozzle is higher than that with straight aperture. In other words, the microsystem becomes more sensitive to smaller loads and microparticles. This higher sensitivity is due to higher velocity and velocity difference at two sides of the aperture. Comparing the velocity results in Table 2-8 and Table 2-5, velocity and velocity difference increase are the reason for this higher deflection.

Table 2-8. The SPMF^3 FEA results with nozzle shape aperture

Fluid	Mass flow rate (g/s)	Velocity at Nozzle (m/s)	Nozzle inlet V_in (m/s)	Nozzle outlet V_out (m/s)	Nozzle inlet P_in (Pa)	Nozzle outlet P_out (Pa)	Deflection (μm)
Water	0.000416	0.008393	0.007414	0.009784	260.67	254.79	15.4

Table 2-9. The SPMF^3 FEA results with diffuser shape aperture

Fluid	Mass flow rate (g/s)	Velocity at Nozzle (m/s)	Nozzle inlet V_in (m/s)	Nozzle outlet V_out (m/s)	Nozzle inlet P_in (Pa)	Nozzle outlet P_out (Pa)	Deflection (μm)
Water	0.000416	0.00789431	0.009155	0.006805	253.4	259.808	26.1

Comparing the SPMF^3 deflection results of the one with nozzle, Table 2-8, versus the one with the diffuser, Table 2-9, shows higher sensitivity when a diffuser is employed. These results are due to higher velocity at the upper stream versus downstream compared to the one with nozzle which has lower velocity at the upper stream versus downstream point.

Nozzle dimension modification can be used to analyze the effects of fluid channel restrictions on the applied force and deflections of the SPMF^3 (Figure 2-11). The change in flow direction applies flow forces that will be modified when the flow passes through nozzle with various restriction dimensions. An FEA modeling with different nozzle dimensions was done and the microcantilever deflections were measured. The results of this simulation are presented in the fifth chapter along with the submitted article.

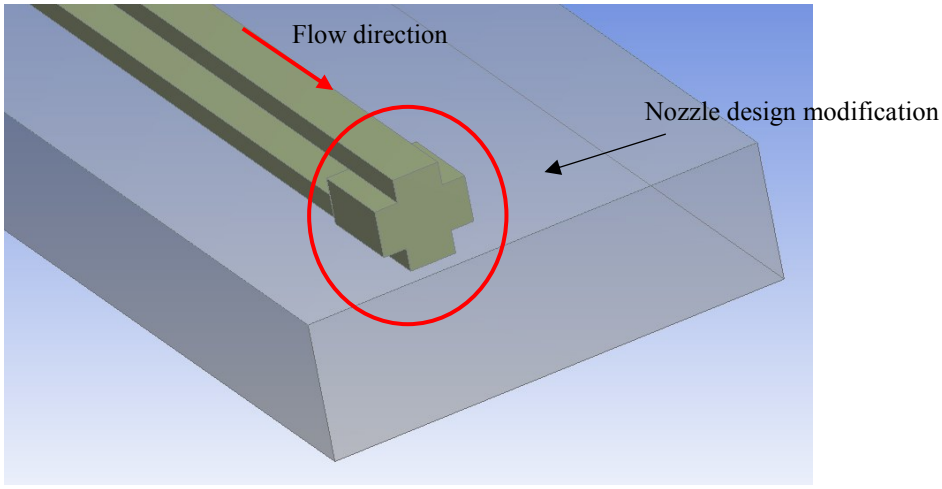


Figure 2-11. Nozzle dimension modification modeling

2.3. Mesh sensitivity analysis

An import analysis that should be done in each FEA modeling setup is mesh sensitivity analysis. In this section, the FEA has been run with different element sizes in order to reduce results sensitivity to the element size. In this problem, two different element sizes have been used to solve fluid dynamics and solid structural equations. The final microcantilever deflection is very sensitive to the fluid problem solution and its element dimension. Thus, an FEA setup has been run with different fluid mesh dimensions and the deflection results against mesh sizes are shown in Figure 2-12.

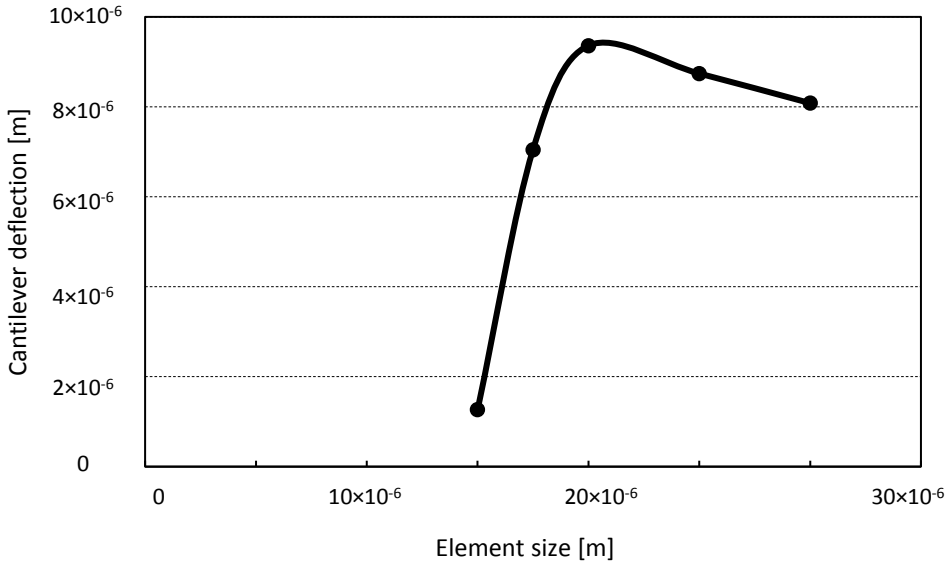


Figure 2-12. Mesh sensitivity analysis results

As it is shown here, increasing the element sizes results in higher cantilever deflection. However, the deflection variation with respect to element size is converging at some element sizes. In other words, the cantilever deflections do not fluctuate and stay in an acceptable region at certain element dimension range. Thus, the final element size that should be set for this modeling is within the converging element range. According to this results, final element size can be in a range of 20 to 30 μm where the cantilever deflection does not change drastically. On the other hand, since the microchannel size is 100×200 μm, it is preferable to have smaller element size possible in the fluid simulation. Finally, 20 μm has been chosen in this FEA modeling to have both fluid and structural simulations in the most accurate situation.

2.4. Conclusions

In this chapter a 3D suspended polymeric microfluidics ($SPMF^3$) concept with a flow plane orthogonal to bending plane is introduced for sensitive biodetection applications. First, a 2D finite element model is presented and feasibility of employing flow forces as a sensing principle is approved. Then, a 3D finite element model coupled with fluid structure interaction is implemented in order to study the $SPMF^3$ behavior and obtain the microcantilever deflection behavior according to dimension variation of microchannel and microcantilever. Finally, the desired microcantilever with an embedded 3D microfluidics has been modeled with different nozzle and diffuser shapes and dimensions. This confirms that the $SPMF^3$ can be designed for different biosensing applications through modification of microchannel and nozzle dimensions.

Chapter 3

Flow Force Augmented 3D Suspended Polymeric Microfluidic ($SPMF^3$) Platform for Sensitive Diagnosis

Detection and study of bioelements using microfluidic systems has been of great interest in the biodiagnostics field. Microcantilevers are the most used systems in biodetection due to their implementation simplicity. These microsystems have been used for a wide variety of applications ranging from cellular to molecular diagnosis. However, increasing further the sensitivity of the microcantilever systems have a great effect on the cantilever based sensing for chemical and bio applications. In order to improve further the performance of microcantilevers, a flow force augmented 3D suspended microchannel is proposed using which microparticles can be conveyed through a microchannel inside the microcantilever to the detection area. In this chapter, the designed microsystem has been analyzed theoretically, simulated and tested. Moreover, the microsystem has been fabricated and tested under different conditions, the results of which have been compared with simulation results.

3.1. Introduction

Mechanical biodiagnostic systems, mainly microcantilevers, have shown high capability in biological studies due to their simplicity and their ability to be microfabricated which is necessary to manipulate biomaterials. Using these microcantilever systems, even femtogram order of limits have been detected and studied. Limit of detection in microcantilever systems is well distributed against analysis time [1]. In other words, an appropriate mechanical microstructure can be employed based on the required detection time. This feature as well as other advantages such as simplicity of employment have made microcantilever a desirable mechanobiological study tool. However, there are some shortcomings such as repeatability, throughput, measurement difficulty, and low quality factor that need to be addressed.

In designing a microstructure, many parameters have to be considered such as size and physical properties of biomolecules, detection limit, throughput limit, need for binding or not, ex situ or in situ, etc. Each of these parameters imposes some criteria on microcantilever size, working medium, quality factor, etc. Based on the aforementioned design parameters, microcantilevers are used in two main ways for biodetection: Stress-based and Frequency-based [53]. Stress based biodetectors measure deflection or bending of the microcantilever exerted by the effects of biomolecules adsorbed on it. However, frequency-based bidetectors measure the shift in resonance frequency when another mass, such as biomolecules, is added to the microcantilever. In terms of complexity of microcantilever devices, one can categorize them in two branches of with or without an external exciter. In other words, frequency-based systems use an external exciter to detect natural frequency changes while stress-based ones do not need an exciter as they detect bending deflection.

3.1.1. Stress-based microsystems

In these microstructures, the surface of microcantilever which is covered by biomolecular receptor is called active side and the other side is called passive side. Biomaterials are binding to the active side and provide either excess mass or surface stress that deflects the microcantilever. This surface stress can reach up to the amount of 0.01 Nm^{-1} which is enough for cantilever deflection in the order of tens of nanometers [54, 55, 56, 57, 58]. If the surface stress is compressive, microcantilever shows downward deflection and if it is tensile, microcantilever shows upward deflection.

Stress based microcantilevers have been widely employed in real-time biomedical applications. Since 2000 when Fritz et al. [59] started using this method for detection of DNA hybridization, various other applications have been introduced. After injection of complementary DNA, hybridization process could be carried out on the cantilever. This will produce a deflection on the affected microcantilever followed by a displacement (Δx) signal which quantifies the amount of detected DNA. Carbohydrates function analysis as another subject was followed by Gruber et al. [60]. These microcantilevers have also been used for pathogen detection which is one of the frequently used experiments during antibiotic developments. Using this method Ndieyira et al. [61] obtained a sensitivity of 10nM (nM stands for nanomolar) to reveal the bacterial resistance to antibiotics.

In cancer detection, Wu et al. [62] detected prostate-specific antigen in concentrations ranging from 0.2 ng/mL to 60 µg/mL. In another experiment, Savran et al. [63] detected very low protein concentrations using microcantilevers functionalized with Oligonucleotide aptamers. Using a simple microstructure setup including two cantilevers as reference and sensor, a low protein concentration of 100 pg/mL was detected. To obtain this sensitivity, microcantilever deflection of 3-32nm caused by surface bending stresses of 1-10 mN/m was measured.

These techniques were followed by many innovations towards producing more stable and reliable microsystems packaged as a test instrument. Furthermore, a concept of making a microcantilever array sensor has also been studied. For instance, Backmann et al. [64] tested a microcantilever array with 8 cantilever sensors. Each cantilever can be functionalized by a different receptor as a result of which different bioparticles can be sensed in one assay. Using this microcantilever, different peptides with sensitivity of 20 ng/mL were detected in this experiment.

3.1.2. Frequency-based microsystems

Adsorbed mass on a microcantilever affects directly its natural frequency. In other words, micro/nanocantilevers can be employed as a mass detector. In order to design a frequency-based microcantilever it should be noted that the size of the biomolecule or particle under investigation is coupled with microcantilever dimensions. In other words, the smaller particle measurement, the smaller micro/nanocantilever required. On the other hand, lesser the particle size/mass, the higher quality factor required. Regarding the size, there are some methods to fabricate cantilevers in nano dimensions. However, nanofabrication techniques are irreproducible in terms of dimensions and mechanical properties [5]. Regarding the quality factor, the bioparticle size defines the required quality factor as a precision parameter. This parameter should be in the order of 1,000-100,000 for micro/nanocantilevers. An impediment to reaching this amount is the medium around microsystems.

In order to deliver biomolecules on a microstructure, a liquid medium is usually employed. However, this medium will drastically decrease the quality factor to the order of 1-10. To overcome low quality factor issues of microcantilevers immersed in fluid, some solutions have been proposed and tested: using other vibration modes and using ex situ method.

Exciting higher vibration modes was implemented by Braun et al. [42] which shows that exciting of the 16th mode instead of the 1st one will increase the quality factor from 1 to 30. In another investigation, Tamayo et al. [43] increased the quality factor in water medium using an active amplification method. In this experiment it was shown that quality factor of microcantilever in liquid medium which is around 1-10 can be increased by three orders up to 1000 through the proposed Q-control method. As a result of this microresonator experiment, a quality factor of 625 in liquid medium was reached and an antibody, STAR71, with concentration of 0.8 μg/mL was detected. Although this technique is practical for quality factor improvement up to 1000, variation of local viscosity due to temperature changes in fluid makes unstable vibrations for higher quality factor values.

Ex situ measurement is a method in which measurement is done in air/vacuum after rinsing the microstructure inside the biomolecule fluid. This method was employed by Ramos et al. [44] for DNA analysis. The main drawback of this method is the risk of contaminations after rinsing which may degrade the measured parameters. Although applying this method is not highly reliable to get valuable results, Craighead et al. [45] measured prostate specific antigen with sensitivity of 50 fg/ml. In this experiment, a microresonator made of silicon nitride using lithography technique was employed. After measuring the natural frequency of resonator in vacuum, it has been functionalized with appropriate receptors to detect prostate antigens. In this step, microstructure is immersed in a diluted mixture of receptors and washed and dried after 90 mins of exposure to the mixture. Finally, resonance frequency is measured by exciting the microstructure on an external piezoelectric element. In another work, Craighead et al. [65] used this technique to detect single cell of E. Coli by measuring frequency changes of a microcantilever in vacuum instead of atmospheric pressure to increase sensitivity of this microsystem to 1.1 Hz/fg.

The high quality factor in microcantilever systems without fluidic medium makes them a promising tool to measure mass of any particle even in molecular orders. Moreover, these microstructures have been widely used in biological detection and experiments. Lee et al. [47] employed such a microcantilever to detect the prostate-specific antigen by measuring the frequency shift. Using this method, samples with concentration of 10 pg/mL were measured. In this experiment two variations of the microcantilevers with different

dimensions of 100×300μm and 50×150μm were employed. The results show that smaller cantilevers have a higher frequency shift when detecting the same concentration of a microparticle. In other words, it was proved that for detection of lower concentrations of any cell/particle, a smaller microstructure is required.

According to the aforementioned studies, to address the fluid medium drawbacks such as the low quality factor, liquid should be replaced with air/vacuum. However, delivery of biomaterials without a fluid should be investigated. Roukes et al. [46] delivered bioparticles using electrospray injection from fluid to vacuum medium. Frequency monitoring shows real-time shifts when each particle hits the nanocantilever. In this experiment, the mass of 66kDa (1.09e-10 ng) of BSA and 200kDa of b-amylase was measured. This method works based on mass spectrometry which ionizes particles and measures the mass to charge ratio. However some molecules are not suitable to be ionized and may be damaged.

The research on microcantilevers sensitivity and reliability have shown a great promise in mechanobiological study of cells. However, delivering cells mixture to the microcantilever needs a fluidic medium which limits sensitivity of the microsystem. This low sensitivity is due to the low quality factor of moving microstructures in fluidic mediums as damping is much higher than air and vacuum. Moreover, evaporation of fluid that happens in surrounded medium of microcantilevers affects the repeatability of the results.

To overcome these drawbacks, suspended microchannel resonator (SMR) method has been introduced and tested [49]. Being designed and fabricated in a compact silicon chip, this microsystem exhibits results with high sensitivity and repeatability. One drawback with this suspended microsystem is its high stiffness since it is made of silicon and microparticles cannot deflect a microcantilever without an external electrostatic excitation. Using this external excitation, frequency change of the suspended microchannel is monitored which results in extraction of biophysical properties of adsorbed or flown cells and bioparticles.

Through monitoring the vibration frequency of microcantilever, SMR platform can measure the mass of cells or molecules in two different ways. First, when the area of microchannel is functionalized with appropriate receptors, bioparticles will be trapped within the microchannel which consequently changes the vibration frequency of the microcantilever.

Second, when particles are just passing through the microchannel without being trapped there, the frequency of microcantilever shifts when the position of the particle varies.

A shortcoming of the SMR system is its low throughput of 10-100pl/s. To achieve high resolution and sensitivity of 1fg, the flow rate inside microchannel is reduced. In such a condition, only 1-10 particles or cells per second can pass through the microchannel. On the other hand, this low throughput makes it possible to measure mass of individual cells. In this experiment, the mass of E. Coli and Bacillus bacteria was measured with high accuracy in comparison with other investigations.

In order to eliminate external excitation to SMR, the technique of fabricating this suspended microfluidic platform out of a polymer such as PDMS has been introduced and tested. SadAbadi et al. [52] designed and tested this new suspended polymeric microchannel (SPMF) which is less stiff than the silicon one. Therefore, cells and bioparticles can deflect the microcantilever with their physical properties such as mass.

Up until now, frequency variations is the transduction principle of the SMR system. However, these microsystems have another aspect which can be used for behavior analysis and detection. This aspect is the momentum change in fluid flow due to change in flow direction or size variation of the suspended microfluidic channel. The resulting microfluidic momentum change will create a force on the suspended microstructure. Monitoring this force due to momentum change as transduction principle has been widely used in commercial flow meters. In this study, a 3D innovative suspended polymeric microfluidic ($SPMF^3$) platform has been designed, simulated and compared with the existing microsystems in terms of its sensitivity. The design concept and a brief comparison among these existing biodiagnostic systems are shown in Figure 3-1. This microsystem holds a microchannel in a perpendicular plane to the neutral plane of microcantilever. This has improved the microcantilever sensitivity. Based on the simulation results, this microsystem is 5 times more sensitive compared to published results under the same testing conditions, hence the possibility of detecting 5 times smaller bioparticles.

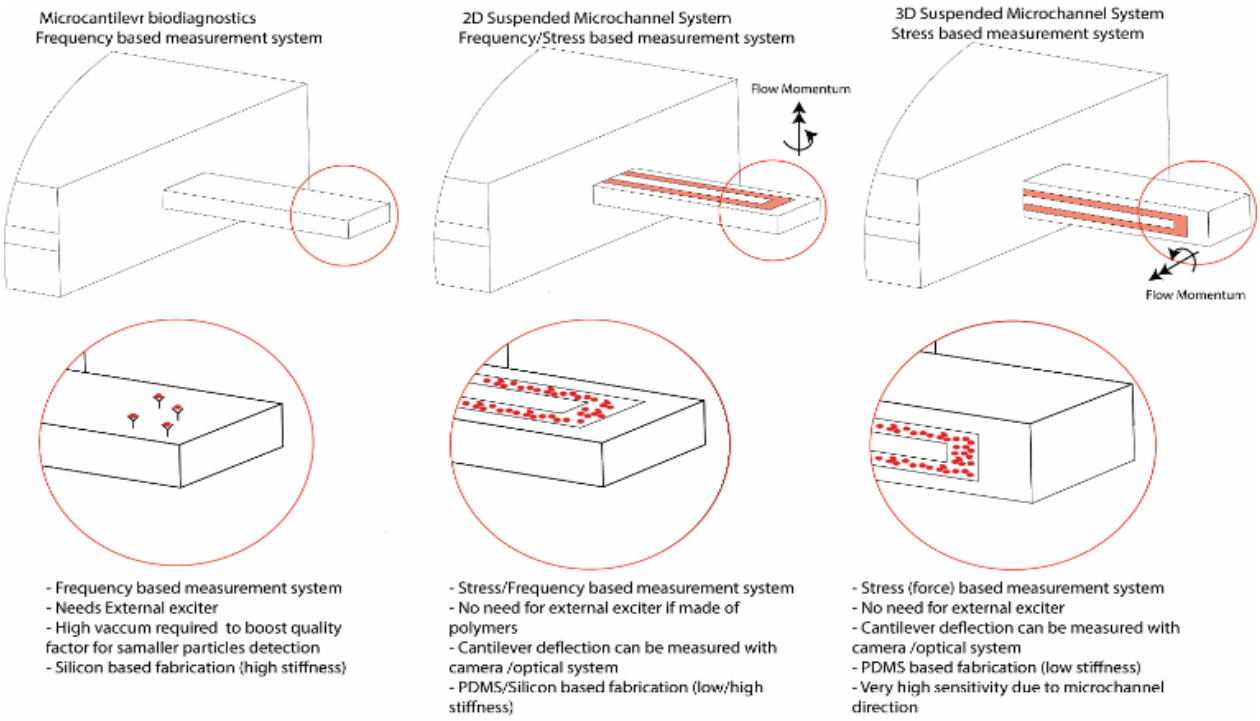


Figure 3-1. Comparing different microcantilevers used for biodiagnostics with 3D innovative suspended microchannel

3.2. 3D Polymeric Suspended Microfluidic Platform (SPMF^3)

3.2.1. Microsystem Design and identification

Suspended Microchannel Resonator (SMR) works based on frequency analysis of the suspended microcantilever while particles pass through the microchannel [49]. However, an external exciter is used to move the microcantilever since the amount of applied force by microparticles is too low to bend the stiff SMR which is made of silicon. This issue was addressed by replacing silicon with PDMS in a technique called suspended polymeric microfluidics by SadAbadi et al. [52]. In this method, a microcantilever with the size of 1.2×4 mm has been designed in which a 15×200 µm microchannel is buried.

The aforementioned silicon SMR and suspended PDMS microsystems have a microfluidic channel in the plane of the microcantilever. In other words, the fluid momentum force created due to microparticles flow are applied perpendicular to microcantilever deflection

direction. Therefore, the current design of suspended microfluidics is unable to detect the forces due to flow change.

Thus, the microchannel configuration has to be modified in a way that sensitivity of suspended microsystem allows this force to be detected. To this end, a new concept of 3D suspended microfluidics has been introduced in this study.

The 3D microchannel inside microcantilever is comprised of three different layers which, bonded together, make the whole suspended microfluidics (Figure 3-2). Changing the microchannel plane to be perpendicular to microcantilever plane, drastically decreases moment of inertia in that direction and increases the sensitivity of the designed microsystem. Furthermore, changing the material of microcantilever from silicon to PDMS will increase the amount of deflection and sensitivity to small loads due to reduced stiffness and Young's modulus. The detail view of the microcantilever tip shows the embedded microchannel and flow direction inside it. The fluid momentum change due to variation in flow direction, geometrical blockage in nozzle area and microparticles flow creates a flow force that acts along the thickness direction of the cantilever. As these forces are along the less bending stiffness plane of the cantilever, they will create more deflection of the cantilever. Monitoring these deflections will provide a new and innovative method to detect and study biophysical properties of cells and flow properties.

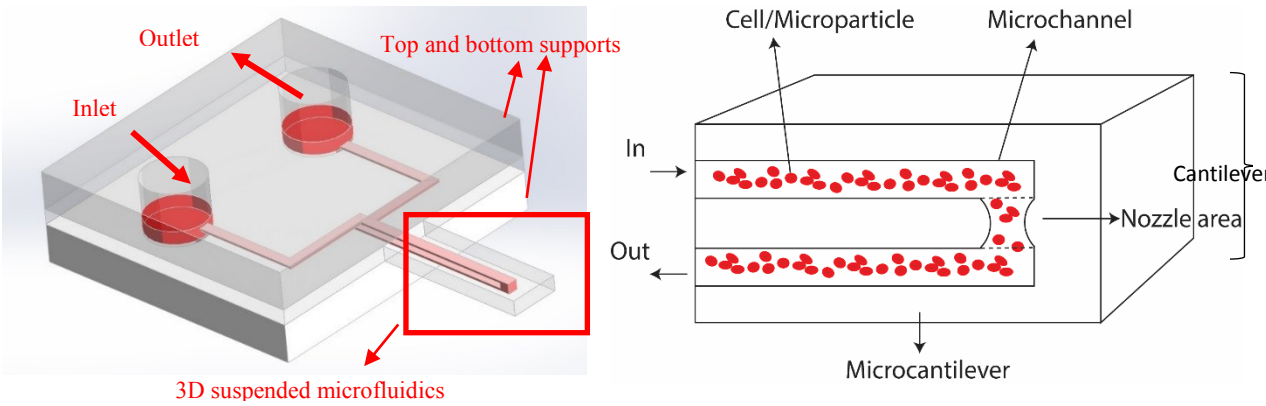


Figure 3-2. Designed 3 dimensional suspended microfluidics

3.3. Microdevice Concept

In this section the flow forces and related parameters will be explained. The main effective forces of suspended microchannel which are applied on the microcantilever are shown in Figure 3-3. These forces are created due to flow direction change and nozzle blockage geometry which finally bend the cantilever tip when flow passes through microchannel. As it is shown here, f_1 and f_2 are the created flow forces at two flow direction change points in microfluidic channel. These forces have two x and y components which can be obtained using the fluid velocity at the points a and b for the f_{1x} and f_{1y} at the first point and, c and d for the f_{2x} and f_{2y} at the second point.

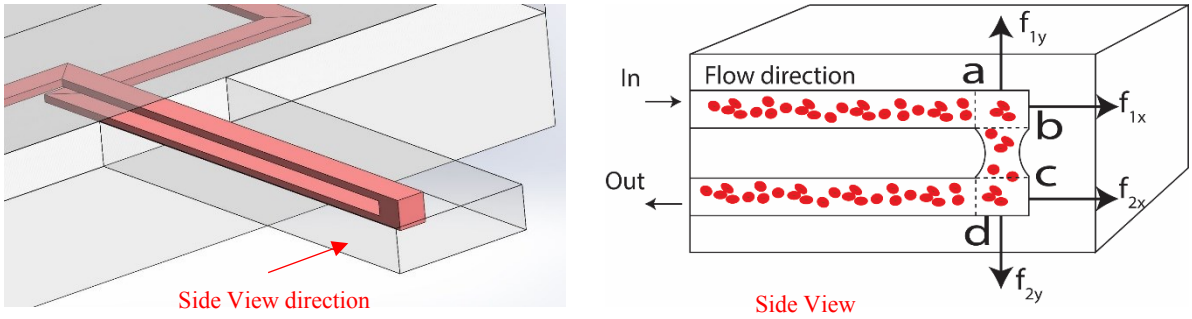


Figure 3-3. Effective forces diagram on the microcantilever

Due to flow losses of the microchannel and nozzle geometry variation, there is a difference between V_b and V_c which results in f_{1y} different from f_{2y} that consequently bends the microcantilever. Thus, microcantilever would bend upward or downward depending on the value of V_b and V_c. The applied flow force equations can be derived as follows:

$$\vec{f_1} = \dot{m}(\Delta\vec{V}), \qquad f_{1x} = \dot{m}(V_a), \quad f_{1y} = \dot{m}(V_b)$$

$$f_{2x} = \dot{m}(V_d), \quad f_{2y} = \dot{m}(V_c)$$

Where $\dot{m}$ is the flow rate inside suspended microchannel.

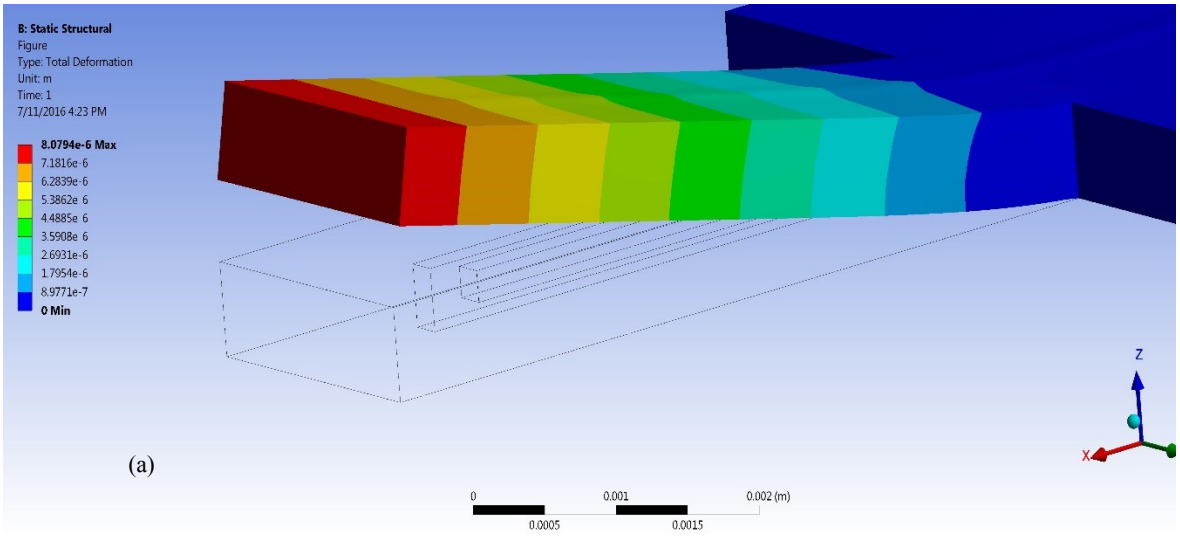
3.4. Detail design

A finite element simulation with rough dimensions has been done as the first iteration. Then, an optimization analysis for sensitivity improvement is done in order to obtain the optimum dimensions. Table 3-1 summarizes few sets of dimensions that have been used for preliminary simulations and the resulting deflection.

Table 3-1. Parameters used for suspended microfluidics simulation and results

Design iteration	Cantilever size, L, W, T (µm)	Microchannel size, W, T (µm)	Nozzle size, L, W (µm)	Speed (mm/s)	Deflection (µm)
1	6000×2000×165	200×15	200×15	10	0.809
2	6000×2000×300	200×50	200×200	30	3.196
3	6000×2000×600	200×100	200×400	30	8.079

Initially, a microchannel with the size of 200×15 µm has been embedded inside a microcantilever with the size of 6000×2000×165 µm, length, width and thickness, respectively. Using the FE simulation, the maximum deflection of microcantilever under flow forces was estimated as in Table 3-1. As expected, the simulation results, agreed with upwards microcantilever deflection according to the forces shown in Figure 3-3. The simulated deflection behavior and fluid structure interaction simulations are depicted in Figure 3-4.



(a)

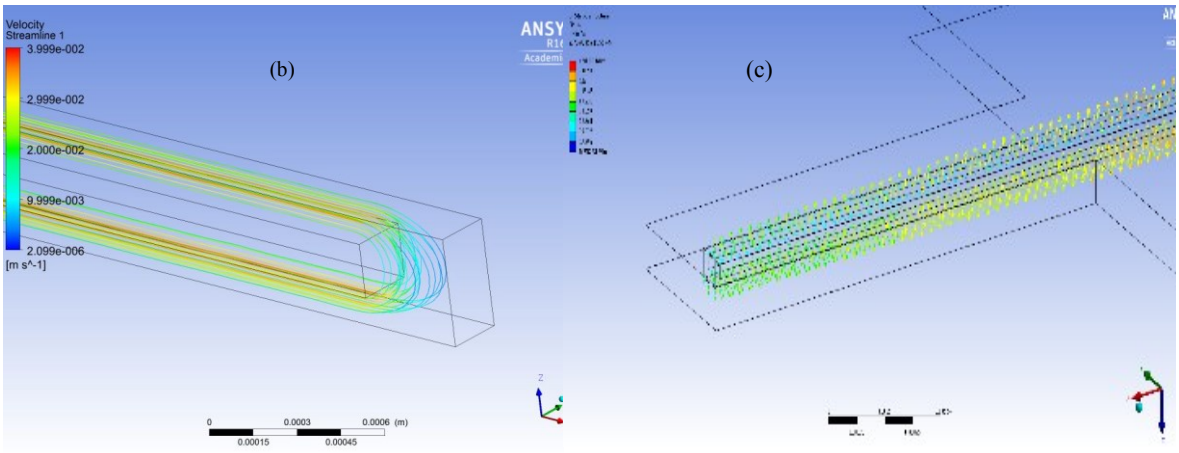


Figure 3-4. a) Deflection under steady flow, b) flow streamline simulation results, c) applied flow pressure to microcantilever

The next step is to increase the sensitivity of SPMF[3] to the applied loads. Here, the microchannel and microcantilever dimensions have been modified so that the amount of microcantilever deflection will be detectable easier using an optical lever method. At the same time the design intention is to keep the channel dimensions small enough so that the microsystem is sensitive to microparticles and cells. Finally, the microsystem dimension that has the highest sensitivity and deflection was chosen for sample fabrication: a microcantilever with the size of 6000×2000×600 µm with an embedded microchannel of 200×100 µm.

To fabricate the 3D polymeric suspended microfluidics with a microchannel in the thickness direction of microcantilever, three polymeric layers are fabricated which are then bonded at the end. These three layers consist of two microchannel layers and a connecting nozzle layer (Figure 3-5). These three elements can be separately designed and optimized to have any desired sensitivity results.

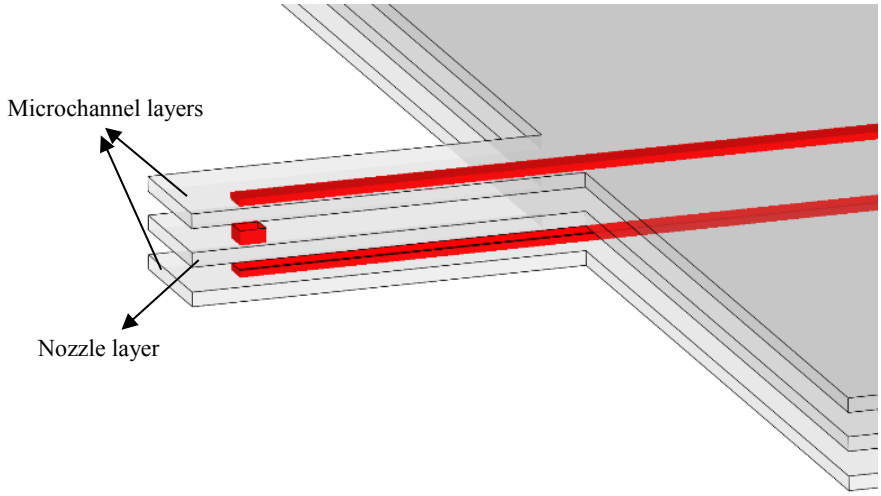


Figure 3-5. Three polymeric layers designed for fabrication of SPMF^3

3.5. Microsystem Fabrication

In this section, a detailed plan for fabrication of SPMF^3 is presented. As mentioned in the design section, two microchannel layers and one nozzle layer are required to be fabricated and bonded together. The microchannel dimensions are chosen based on the detail design results. However, the nozzle dimensions were modified to 400×400 µm to enhance the alignment process during layers bonding step.

3.5.1. Mold making procedure

To fabricate each of the SPMF^3 layers, a proper mask and mold were designed and fabricated. Three different masks are designed using a CAD modeling software (Figure 3-6). Soft lithography was employed to fabricate the three layers. In this study, SU8-2075 used for making the two different required molds: one for the microchannel layers and the other one for the nozzle layer.

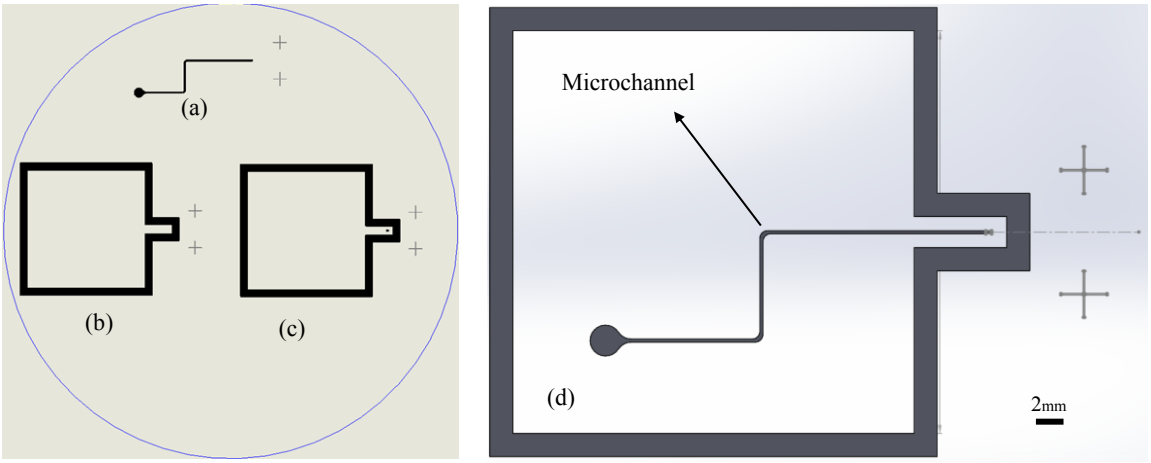


Figure 3-6. Different designed masks using a CAD modeling; a) Microchannel mask, b) Microcantilever mask, c) Nozzle mask, d) whole mask stack up

The microchannel mold is comprised of two features with different thicknesses, one for the microchannel (100µm) and another one for the microcantilever borders (200µm). First, a clean silicon wafer is coated with SU8 using a spin coating machine up to the microchannel depth. Then, microchannel mask (Figure 3-6,a) is employed to be patterned on the coated silicon wafer using a UV exposure machine. After a short post exposure curing on a hot plate, a second layer of photoresist is spin-coated on the first layer. This step is done to cover the microchannel layer when PDMS is poured in mold during layer fabrication. The frame mask (Figure 3-6,b) is prepared to pattern the architecture of microcantilever borders on photoresist in this step. Therefore, the fabricated layer comes out of the mold easier with the exactly designed dimensions during fabrication steps. The cross signs on the masks are used in order to align masks A and B accurately on top of each other during mold fabrication process (Figure 3-6). Furthermore, a separate mold is fabricated for nozzle layer using the designed mask (Figure 3-6,c). In this step, a separate silicon wafer is used since the nozzle mold is patterned in one step using its mask. The entire mold fabrication process is summarized in a schematic view (Figure 3-7,a).

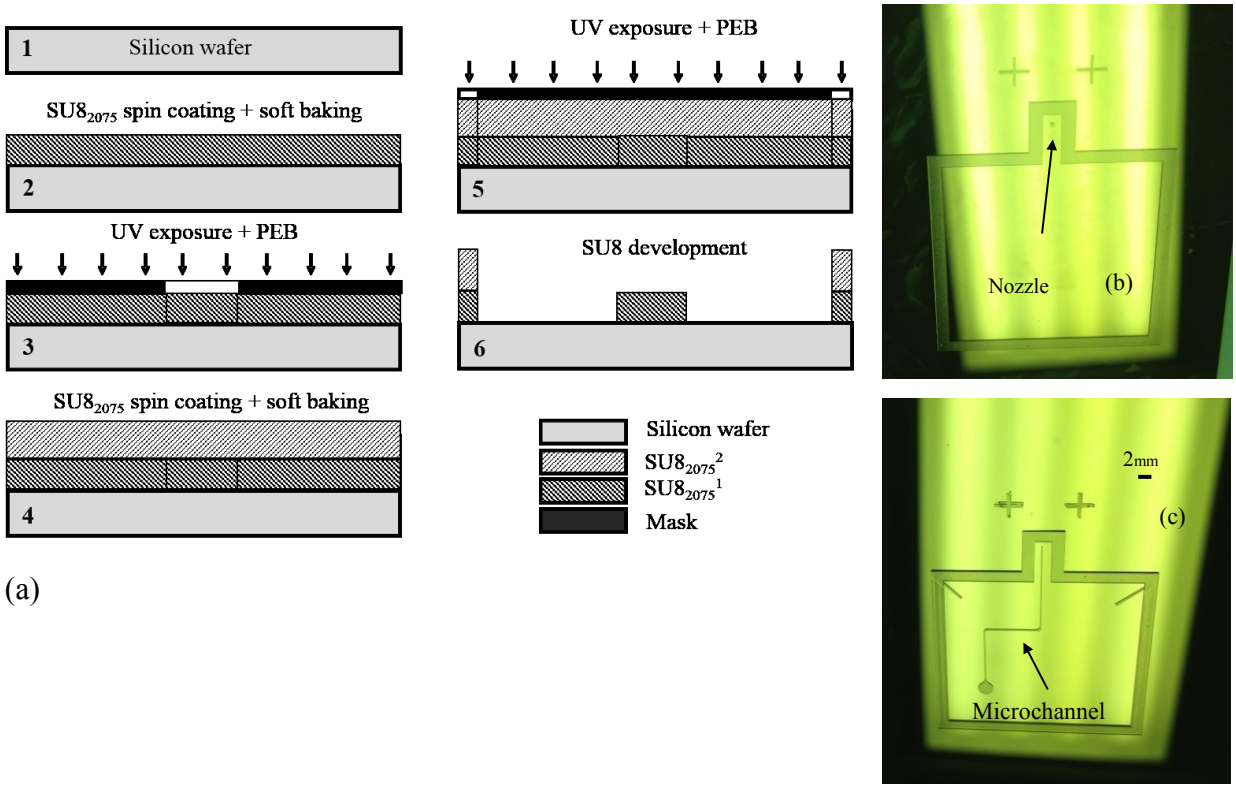


Figure 3-7. a) Schematic of the soft lithography mold fabrication process, b) Fabricated Nozzle mold, c) Fabricated Microchannel mold

Finally, all the patterned and cured molds will be developed inside a photoresist developer solution to remove un-patterned areas from the molds (Figure 3-7,b, c). In order to increase the mold strength and maintain the microsystem fabrication quality during frequent employments of the mold, a hard baking process is recommended. The steps in mold making procedure are summarized in the following Table 3-2.

Table 3-2. Mold fabrication and curing cycle

1	Spin coating using SU8-2075 for 100µm thickness
2	Pre-exposure baking at 65C for 5min
3	UV patterning using microchannel mask
4	Post-exposure baking at 65C for 5 min
5	2nd layer coating SU8-2075 for 100µm added thickness
6	Pre-exposure baking at 95C for 15min
7	2nd UV patterning using cantilever mask

8	Post-exposure baking at 95C for 15min
9	SU8 Developer for 10min
10	Hard baking at 200C for 10-20min
11	Silanization at 65C for 2 Hrs

The polymer used for the SPMF^3 fabrication is PDMS which is mixed with the curing agent with 10:1 ratio. After preparing PDMS and degasifying it, the polymer will be poured into the mold. In order to achieve the predesigned thickness of final PDMS layer, a microscopic glass is used to apply pressure on the mold (Figure 3-8). As it is shown in this figure, the microsystem boundary made in the mold will act as a wall around microsystem limiting the thickness of the final PDMS layer. To maintain this thickness during the curing process, a gripper which fits into oven is employed. Finally, the mold is kept in the oven preheated to 65C for 2 hours.

After fabricating each layer using PDMS, these layers are bonded together to make the final suspended microfluidic system. In this step, each layer is preprocessed in a plasma treatment machine and bonded carefully using a microscope to reach the required alignment accuracy. First, the two microchannel and nozzle layers are cleaned and placed inside the plasma machine. After 30-40 seconds of treatment, these two layers are accurately aligned to form half of the microsystem. These two layers are placed on the glass slips used during fabrication process (Figure 3-8). Thus, when the bonding is done the glass slip of the nozzle layer has to be removed. Similarly, the second microchannel layer is bonded to these two layers. At the end, both glass slips on two sides of the 3-layer microfluidics are removed and proper supporting layers are bonded to hold the microsystem. This process is summarized in the following schematic (Figure 3-8).

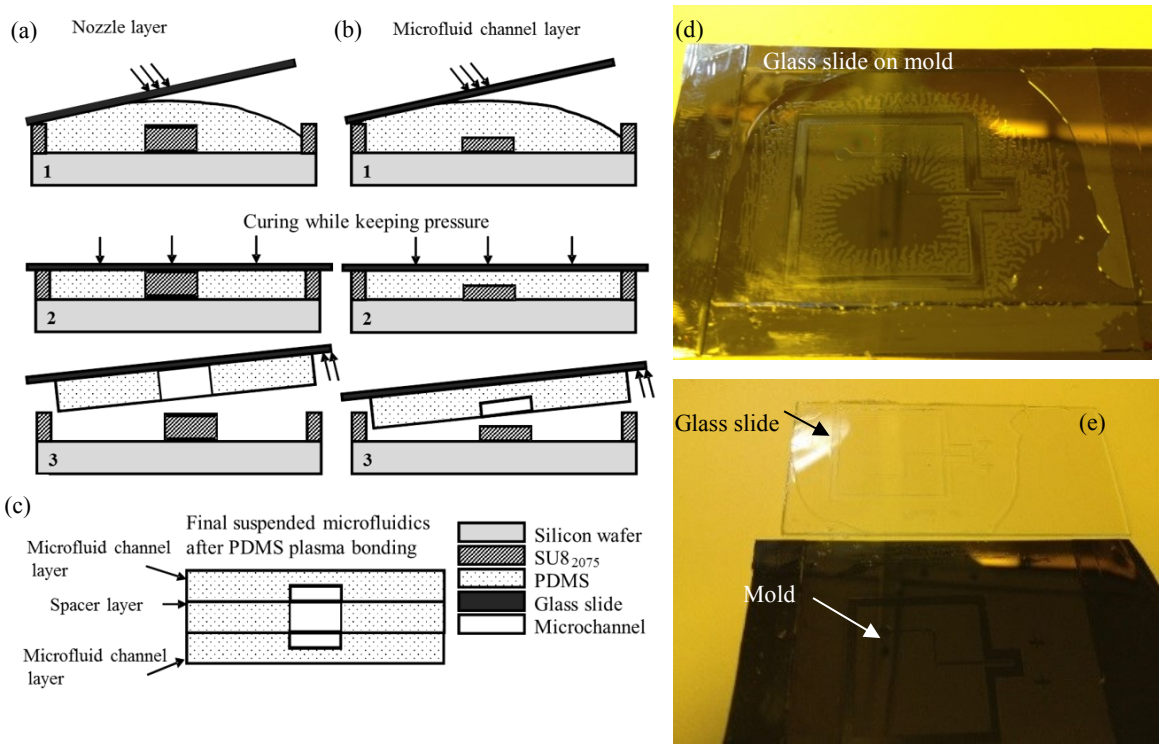


Figure 3-8. Schematic of PDMS layers fabrication for microchannel and nozzle a) Nozzle layer fabrication b) Microchannel layer fabrication c) Bonding procedure d) fabricated layer on the mold after bringing out of oven, e) fabricated layer on glass slip after removing from mold

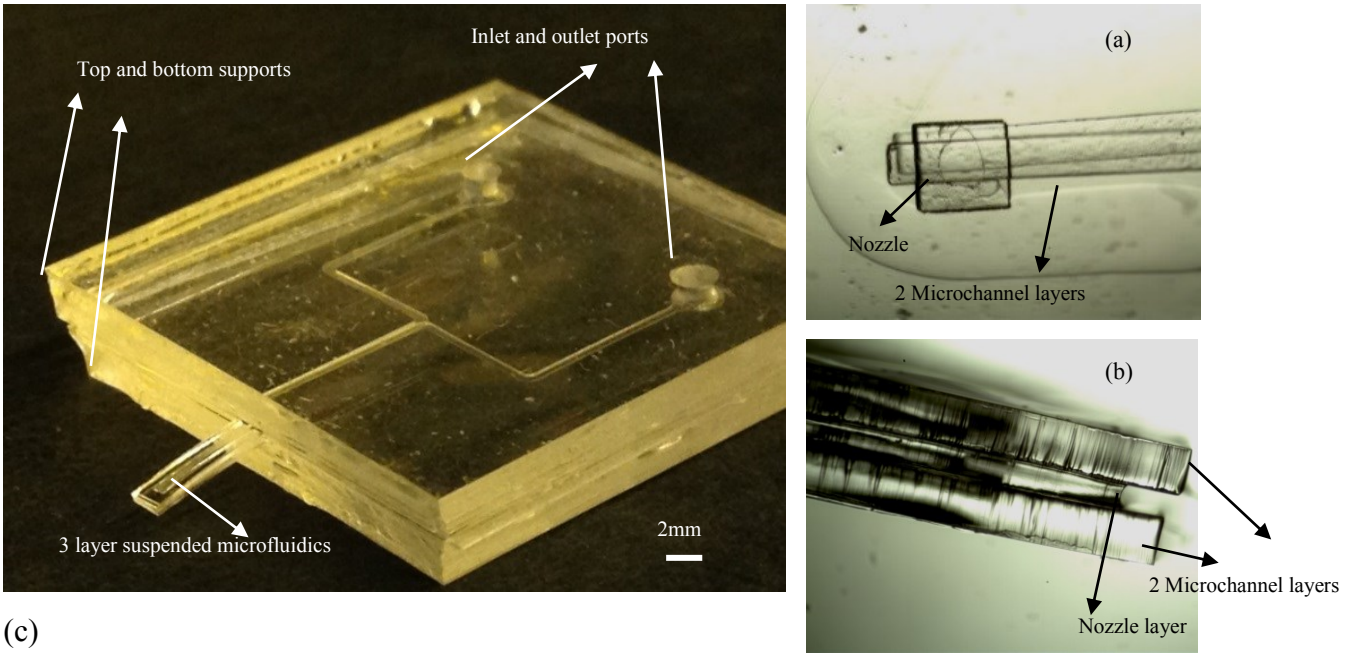


Figure 3-9. The 3D suspended microsystem SPMF[3] a) Top view of microchannel with nozzle b) Side view of microcantilever c) whole microsystem and its input and output ports

In order to maintain functionality of the SPMF^3 as designed and simulated previously, it is crucial to monitor alignment accuracy of these 5 layers during the bonding steps. To this end, the entire process is done under a wide working range microscope. Finally, the suspended microfluidics is ready for flow detection experiments (Figure 3-9). After fabricating the first few microsystems, it was diagnosed that the alignment process in bonding steps is very difficult and time consuming due to nozzle and microchannel dimensions. Thus, the nozzle dimensions were modified from 200×200μm to 400×400μm to enhance the process.

3.6. Sensitivity Simulation and Experiment

In this section, the SPMF^3 platform is compared with a suspended microchannel resonator (SMR) type microsystem in terms of their sensitivity to the same working conditions. Here, viscosity and density have been used as common measures to compare the sensitivity of the discussed microsystems. For this simulation, the input flow rate has been kept the same while viscosity and density change according to Table 3-3. Later in another simulation the sensitivity of these two microsystems is compared in terms of changes in flow rate. Changing the fluid flow will modify fluid speed inside the microchannel which results in changes in applied momentum force and consequently cantilever deflection.

Table 3-3. Initial conditions for sensitivity simulation

Flow rate (μL/s)	Re	Speed (mm/s)	Viscosity (kg/ms)	Density (kg/m^3)	Fluid
50	1-3*	25-35*	0.90×10^{-3}-1.50×10^{-3}	1×10^{3}-1.5×10^{3}	Water

* Changes in viscosity results in Re and Velocity change.

The first simulation was done to obtain the results of a 2D suspended microfluidics (SPMF), designed by SadAbadi et al. [52], with the microchannel size of 200×100 μm, length and width respectively, buried parallel to the neutral plane inside a microcantilever with the dimensions of 6000×1000×600 μm, length, width and thickness respectively. In this simulation, the microcantilever material is PDMS with Young's modulus of E= 700 kPa and density of 970 kg/m^3. The results in Figure 3-10 show microsystem sensitivity to variations of fluid properties. Then, a 3D suspended microchannel with the same dimensions as

previous one, aligned perpendicular to the neutral plane, and nozzle size of 200×200 µm is simulated. The simulation results are shown in Figure 3-11.

The sensitivity of these two microsystems with an embedded microchannel were compared based on three different fluid properties: the fluid density, viscosity and flow rate. The results shown in Figure 3-12 confirm the higher sensitivity of SPMF^3. Since the microchannel of SPMF is in the plane of microcantilever, the flow loads are hence applied in the stiffest direction which lowers microsystem deflection and consequently its sensitivity.

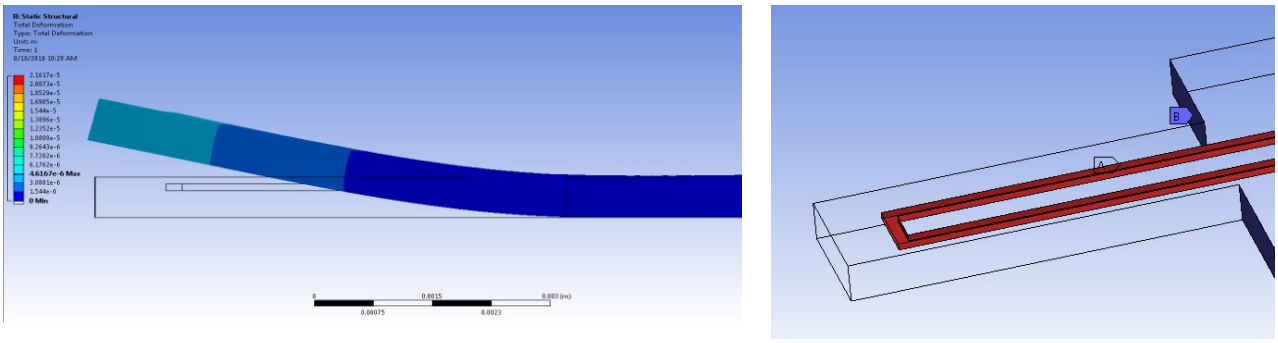
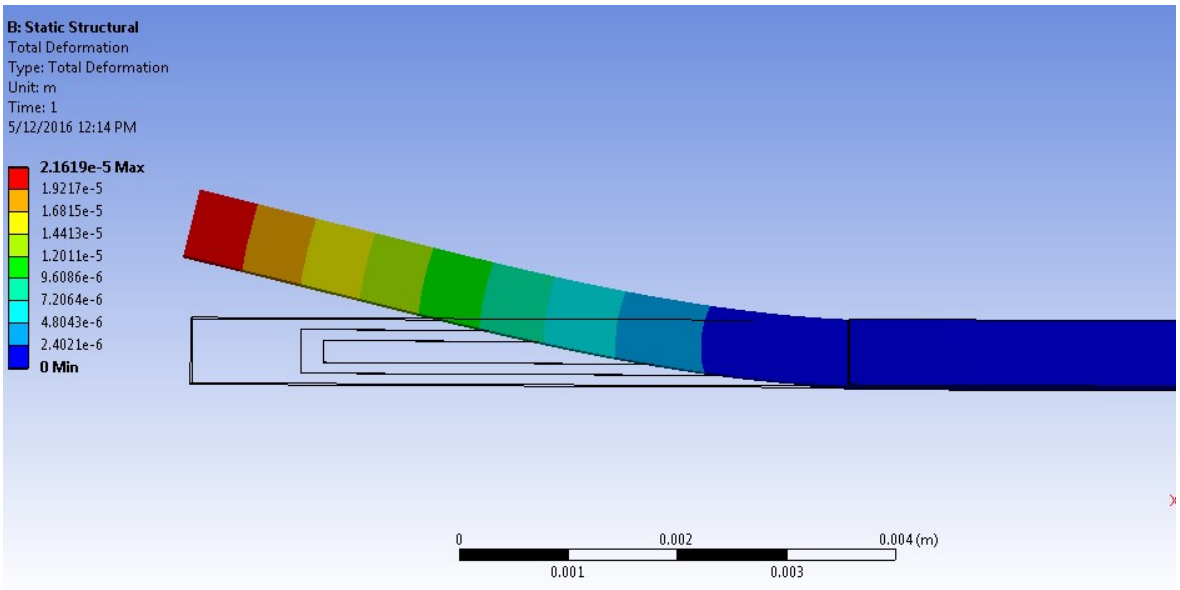


Figure 3-10. Simulation results of 2D suspended microfluidics (SPMF)



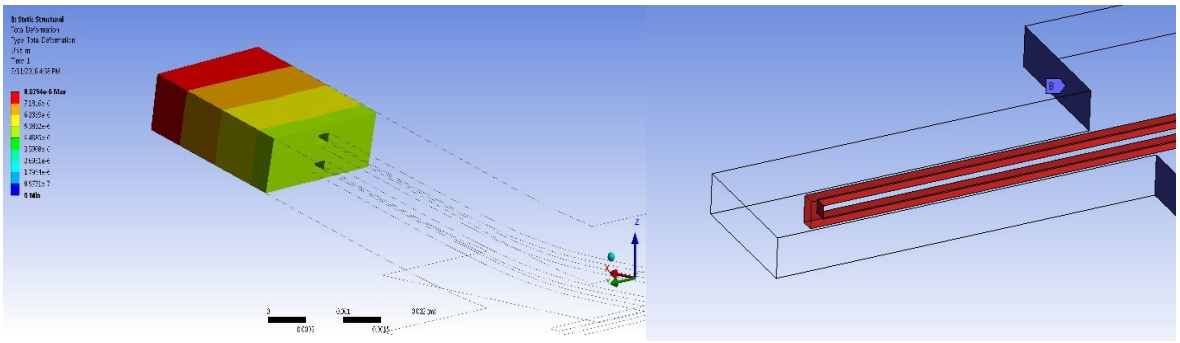
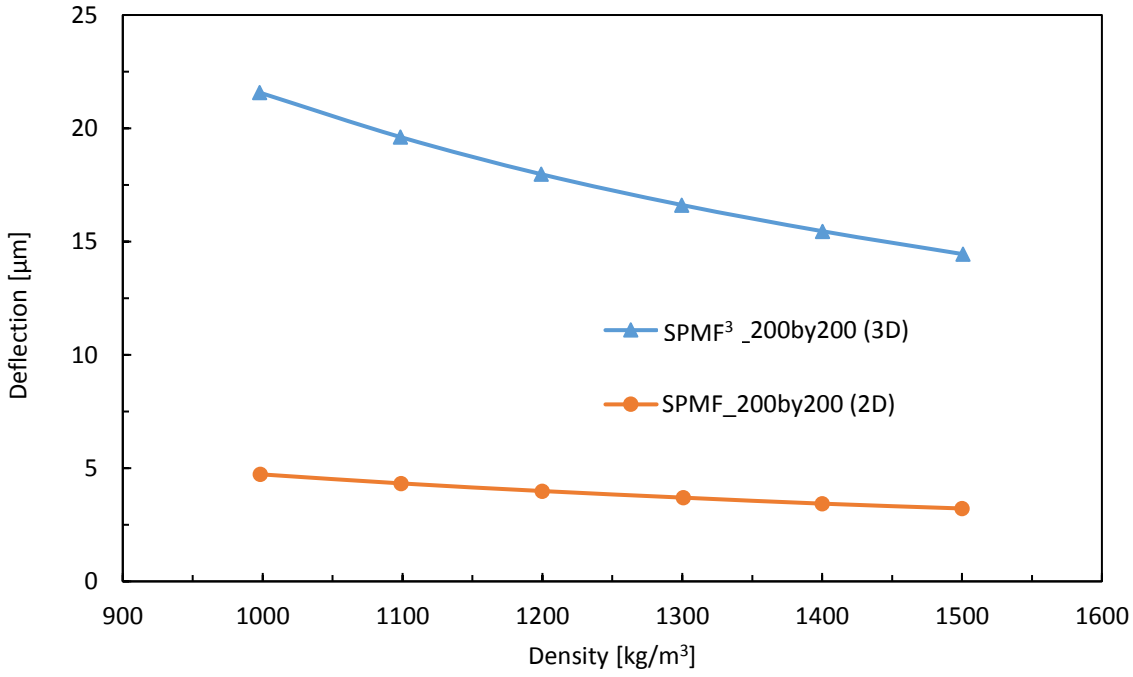


Figure 3-11. Simulation results of the innovative 3D suspended microfluidics ($SPMF^3$)

As mentioned in the device design section, flow and momentum loads can deflect the microsystem through which different fluid properties can be detected. The simulation results show that the higher fluid viscosity the higher cantilever deflection. This effect is reversed for fluid density changes which is due to a higher mass flow rate inside the suspended microchannel and the upward deflection direction.



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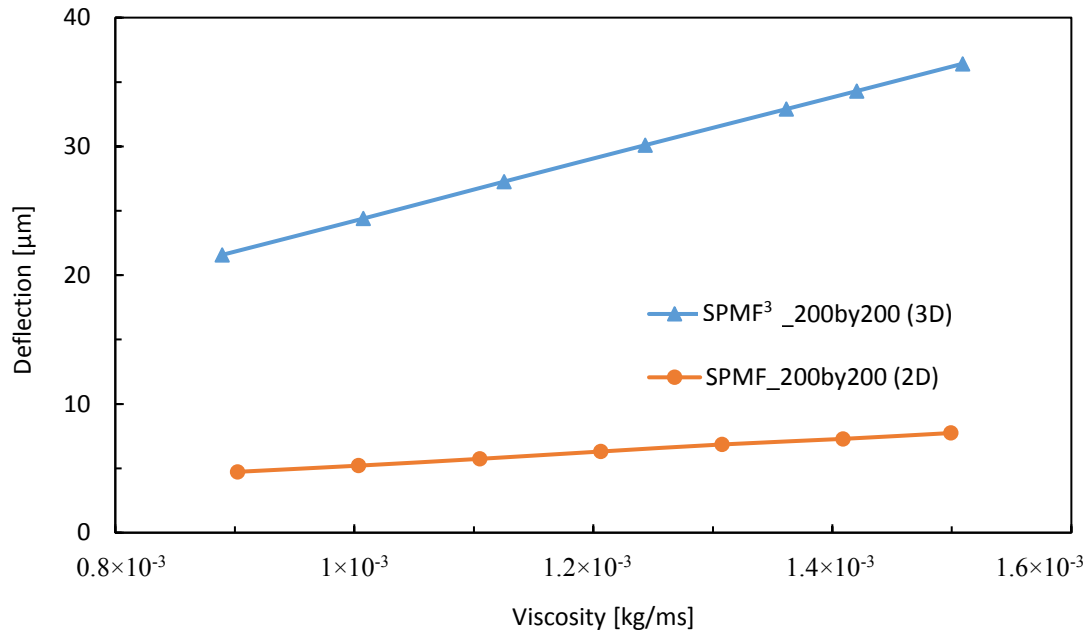

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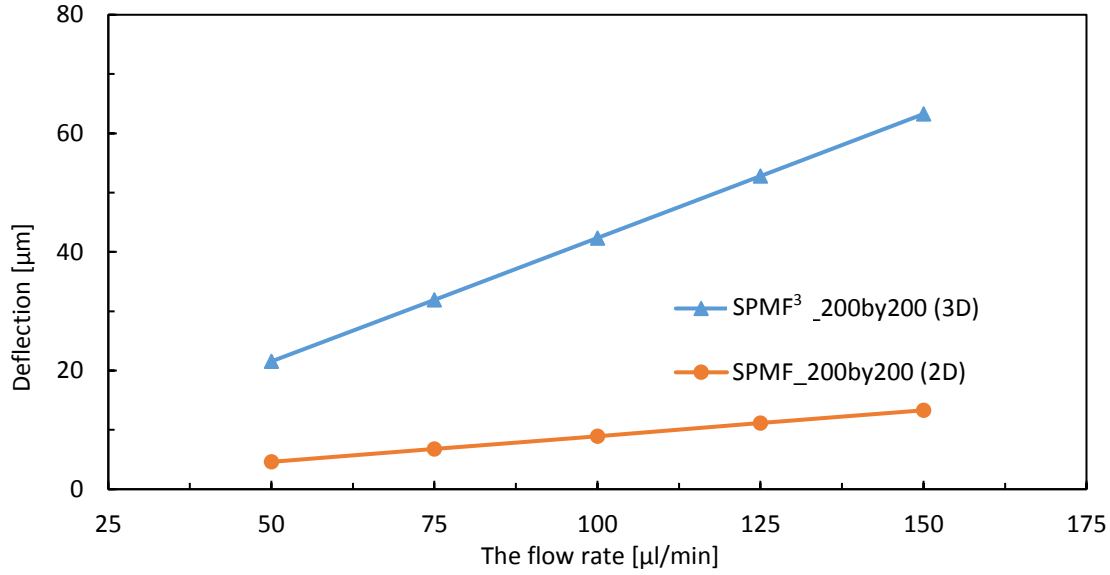


(c)

Figure 3-12. Comparison of SPMF and SPMF^3 microcantilever deflections versus changes in fluid properties based on fixed nozzle dimensions (200×200 µm) a) density b) viscosity c) flow rate changes

Finally, comparison of the simulation results of the 2D and 3D suspended microsystems shows that the later has almost 5 times more sensitivity. This will result in simpler and less expensive biodevices which do not need external exciter and complicated data acquisition systems.

So as to validate the sensitivity of fabricated 3D suspended microchannel to different flow rates, an experiment was performed and the results were compared with simulations. An optical laser based deflection measurement system has been used to detect microcantilever deflections (Figure 3-13).

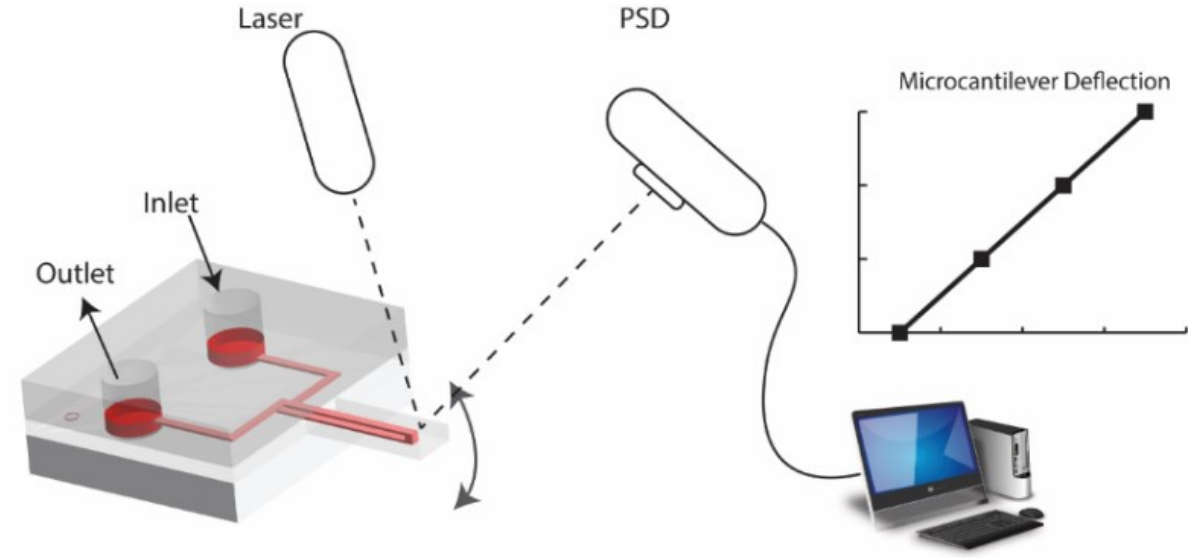


Figure 3-13- Laser displacement measurement system

In this experiment, DI water with different flow rates was inserted into microsystem using a syringe pump (Figure 3-14). Comparing the simulation and experimental results of the suspended microchannel with channel and nozzle size of 200×100 μm and 400×400 μm respectively, shows a good agreement between the model and experiments.

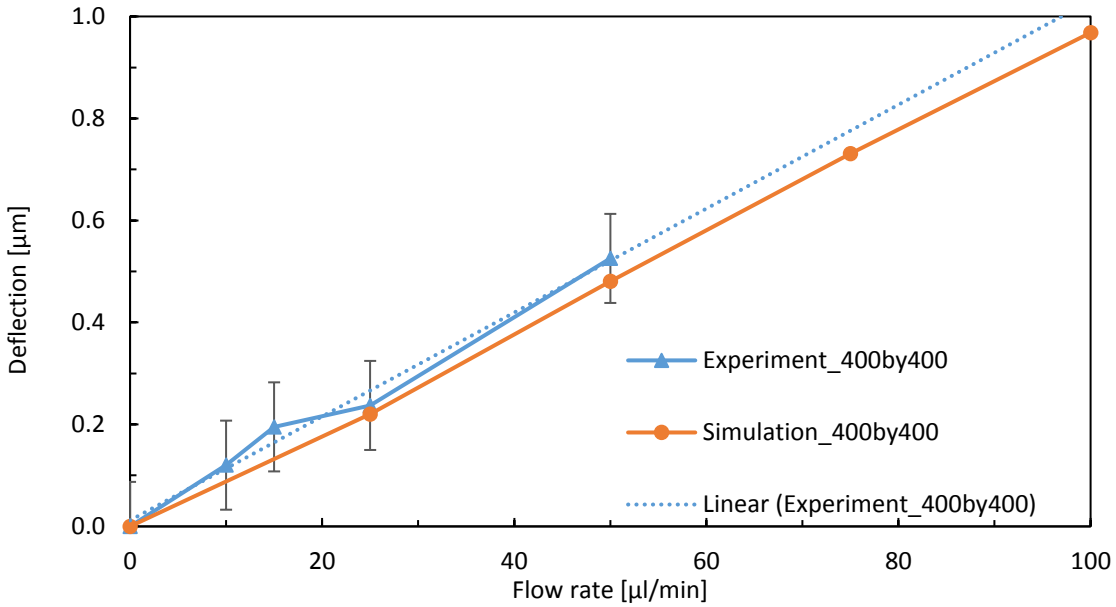
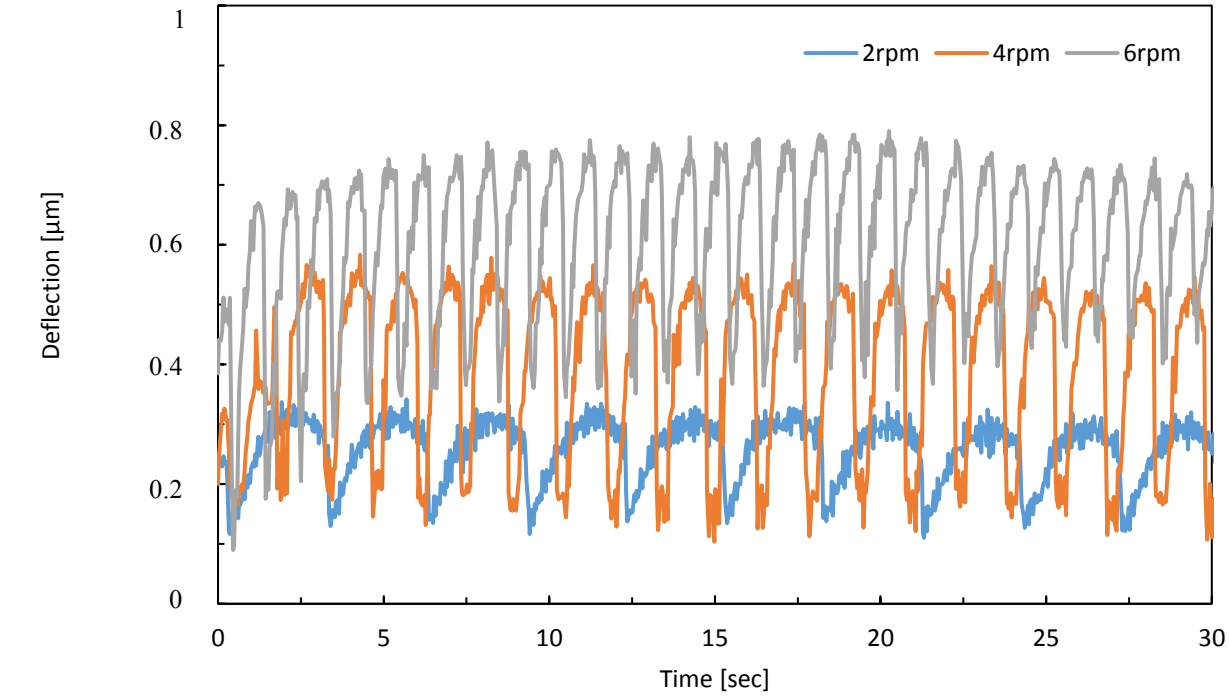
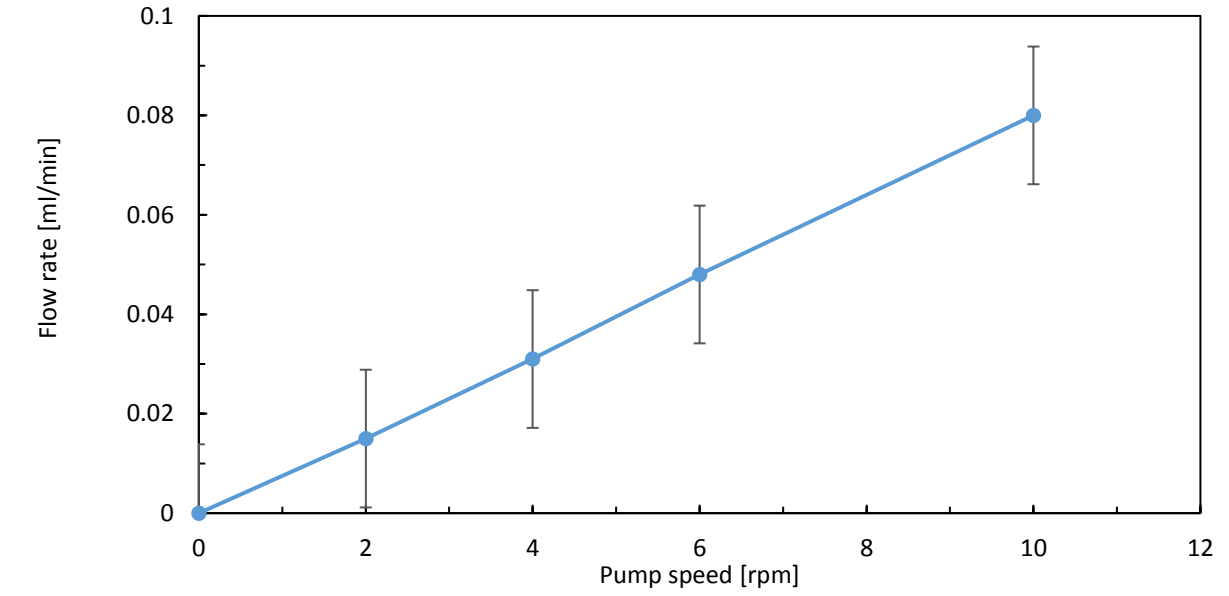


Figure 3-14. Sensitivity experiment of 3D suspended microcantilever and its comparison with simulation results

To further investigate the sensitivity of fabricated SPMF[3] under dynamic loads, another experiment was done using a peristaltic pump. The microcantilever deflection was measured under this harmonic fluid flow with different flow rates as the pump speed changed. As the results confirm, there are different microcantilever response frequencies at different pump speeds since this pump is trigged by the rotating rollers in the pump (Figure 3-15a). The peristaltic pump has 10 rollers and different pump speeds make different fluid flow rates and frequencies in microchannel (Figure 3-15b). Here, the microfluidic system was tested with 2, 4 and 6rpm pump speeds generating flow pulses of 0.33, 0.66 and 1 Hz.



(a)



(b)

Figure 3-15. a) 3D suspended microchannel response to dynamic load by a Peristaltic pump b) The peristaltic pump flow rate versus speed

In order to validate the simulation results which show the sensitivity of 3D suspended microchannel to changes in the viscosity and density of fluid flow, another experiment was conducted. DI water with different concentrations of salt with the intention of varying fluid density and viscosity was used as the fluid in the experiment.

Table 3-4. The 3D microfluidics behavior against variations in fluid properties

Salt wt %	Density (kg/m^3)	Viscosity (cP)	Kinematic Viscosity (cSt)	Deflection (μm)
0 %	999	1.002	1.00	0.48
10 %	1070	1.193	1.11	0.72
15 %	1110	1.350	1.22	1.05

According to the experimental results (Table 3-4), addition of salt can be detected using this microsystem; however, both density and viscosity increase when salt is added to water. Thus, the source of this change can be either one of these parameters or both. The former sensitivity simulation results (Figure 3-12) show that the microcantilever deflection increases when viscosity increases and it decreases when density increases.

In order to compare the simulation and experimental results, kinematic viscosity was used to involve both viscosity and density ($\upsilon=\mu/\rho$). The microsystem deflection results from simulation of suspended microchannel with channel size of 200×100 μm and nozzle size of 400×400 μm is presented versus kinematic viscosity and compared with experimental results in Figure 3-16. As seen in this figure, there is a good agreement between simulation and experiment results which verifies the model that is used for $SPMF^3$ design and development.

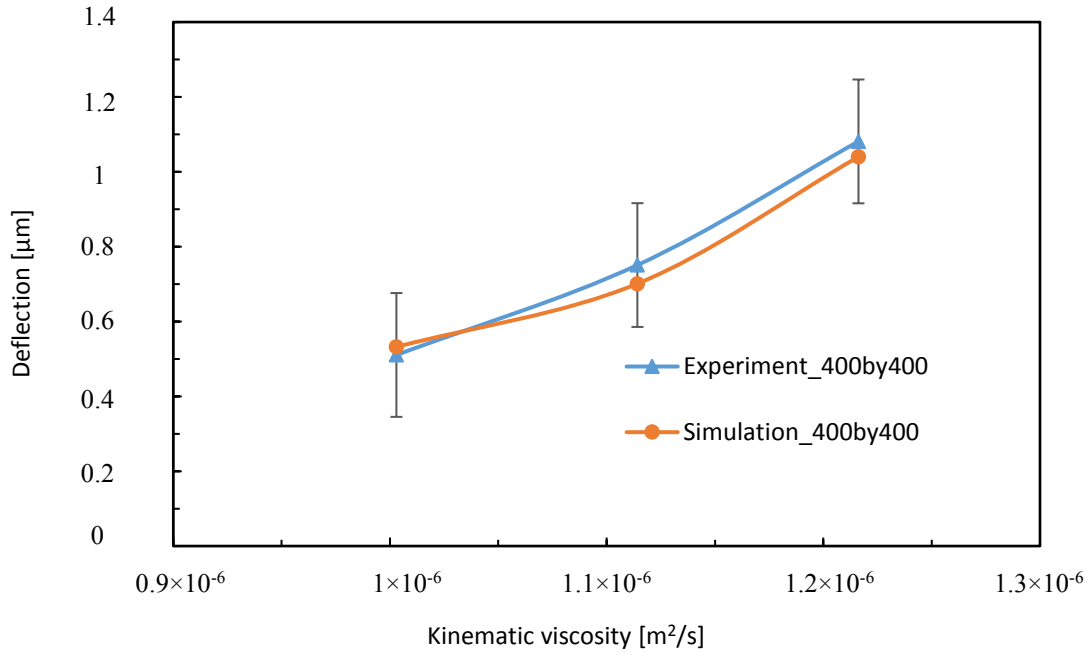


Figure 3-16. Comparison of experimental and simulation results of the 3D microsystems under the same condition

3.7. Conclusions

An innovative 3D polymeric suspended microfluidic concept has been introduced, simulated and tested in this study. The drawbacks of microcantilever diagnostics such as repeatability and the delivery of bioelements to the detection field are addressed using this concept. The need for an external exciter in such microcantilever biodetectors is removed with replacing silicon with PDMS in fabrication process. Furthermore, having used fluid flow forces as transduction principle increases the microcantilever sensitivity in a way that final deflections are measurable with an optical system.

In order to achieve the advantages of the SPMF^3 concept, a polymeric multi-layer fabrication process was designed and implemented through which bonding alignment and strength issues were addressed. A detailed model was presented to assess and compare the designed suspended microfluidics. According to the simulation results, fluid properties such as density, viscosity and flow rate are measurable using the SPMF^3. In addition, an increase of 5 times in sensitivity of the SPMF^3 compared to the similar microcantilevers was achieved.

Finally, the model was validated with experimental results of the fabricated SPMF^3. DI water with different salt concentrations was injected in the suspended microfluidics and the microcantilever deflection results versus variations of fluid properties were measured and compared with simulations.

Chapter 4

3D Suspended Polymeric Microfluidics ($SPMF^3$) with Flow Orthogonal to Bending (FOB) for Fluid Analysis through Kinematic Viscosity

Measuring of fluid properties such as dynamic viscosity and density have tremendous potential for various applications from physical to biological to chemical sensing. But, it is almost impossible to affect only one of these properties as dynamic viscosity and density are coupled. Hence, this paper proposes kinematic viscosity as a comprehensive parameter to study the effect of fluid properties applicable on various fluids from water to non-Newtonian fluids such as blood. This paper also proposes an ideal microplatform, namely, polymeric suspended microfluidics ($SPMF^3$) with flow plane orthogonal to the bending plane of the structure, along with tested results on various fluids covering the engineering applications range. Kinematic viscosity, also called as momentum diffusivity, considers changes in both fluid intermolecular forces and molecular inertia which defines dynamic viscosity and fluid density, respectively.

In this study, a 3D suspended polymeric microfluidic systems ($SPMF^3$) was employed to detect changes in fluid parameters such as dynamic viscosity and density during fluid processes. Using this innovative design along with theoretical and experimental results, it is shown that, in fluids, the variations of fluid density and dynamic viscosity are not easily comprehensible due to their interconnectivity. Since any change in fluid will affect both density and dynamic viscosity, measuring both of them is necessary to identify the fluid or process status. Finally, changes in fluid properties were analyzed using simulation and experiments. The experimental results with salt-DI water solution and milk with different fat

concentrations as a colloidal fluid show that kinematic viscosity is a comprehensive parameter that can identify the fluids in a unique way with the proposed microplatform.

4.1. Introduction

Fluid density and viscosity result in inertia or resistance to move or flow. If we assume that fluid molecules are placed in multiple sheets sited on each other, viscosity is defined as friction force between these sheets. Based on the application and fluid properties, two types of fluid viscosity called dynamic and kinematic viscosity are defined. Dynamic viscosity is mainly used for comparing different fluids from their flow resistivity point view. This resistance comes from the Van der Waals force among molecules of fluid layers. The more force which comes from electron cloud density of atoms makes the more viscosity effect of fluid flow. The fluid molecules have a specific mass which comes to effect when kinematic viscosity is being employed. The higher molecule mass makes more inertia at the molecular level thus, the less tendency (or more inertia) to move and transfer momentum among fluid layers.

The fluids with their viscosity dependent on shear rate are called non-Newtonian fluids and the rest are defined as Newtonian. As an instance, blood viscosity changes with shear rate which means that higher viscosity in capillaries and lower viscosity in larger veins [66]. In other words, an external force applied to non-Newtonian fluids affects their viscosity and shear rate [67, 68]. In order to interpret this non-Newtonian effects, a deeper understanding of kinematic viscosity is required. The non-Newtonian behavior is mainly due to structural organization of molecules inside the fluid. For example, in colloidal fluids such as blood, the shear thinning behavior is because of segregation of particles and phases inside fluid. Thus, connection in phases inside a non-Newtonian fluid will increase the fluid viscosity and density in a control volume such as capillary. Here the definition of kinematic viscosity, $\upsilon = \mu/\rho$, comes to effect in a way that connection among phases inside a fluid not only changes the distance between them and their interaction forces but also the fluid density in a control volume. Thus, kinematic viscosity varies with shear stress in non-Newtonian fluids.

As such adding or removing substances from a fluid, reorganize atomic arrangements inside fluid leading to changes in both dynamic viscosity and density. As it is shown in Figure 4-1, the substance atoms are attracted by existing solvent atoms. This creates new Van der Waals forces among fluid molecules which result in formation of new molecules and consequently higher friction or viscosity [69]. At the same time, molecular weight is modified due to introduction of new atoms and creation of new molecules. The higher molecular weight results in the higher fluid density. As mentioned, any variation in fluid substances during a process affects both density and viscosity therefore, another comprehensive parameter which considers both is required to specify and study the fluid in a more comprehensive way during different processes.

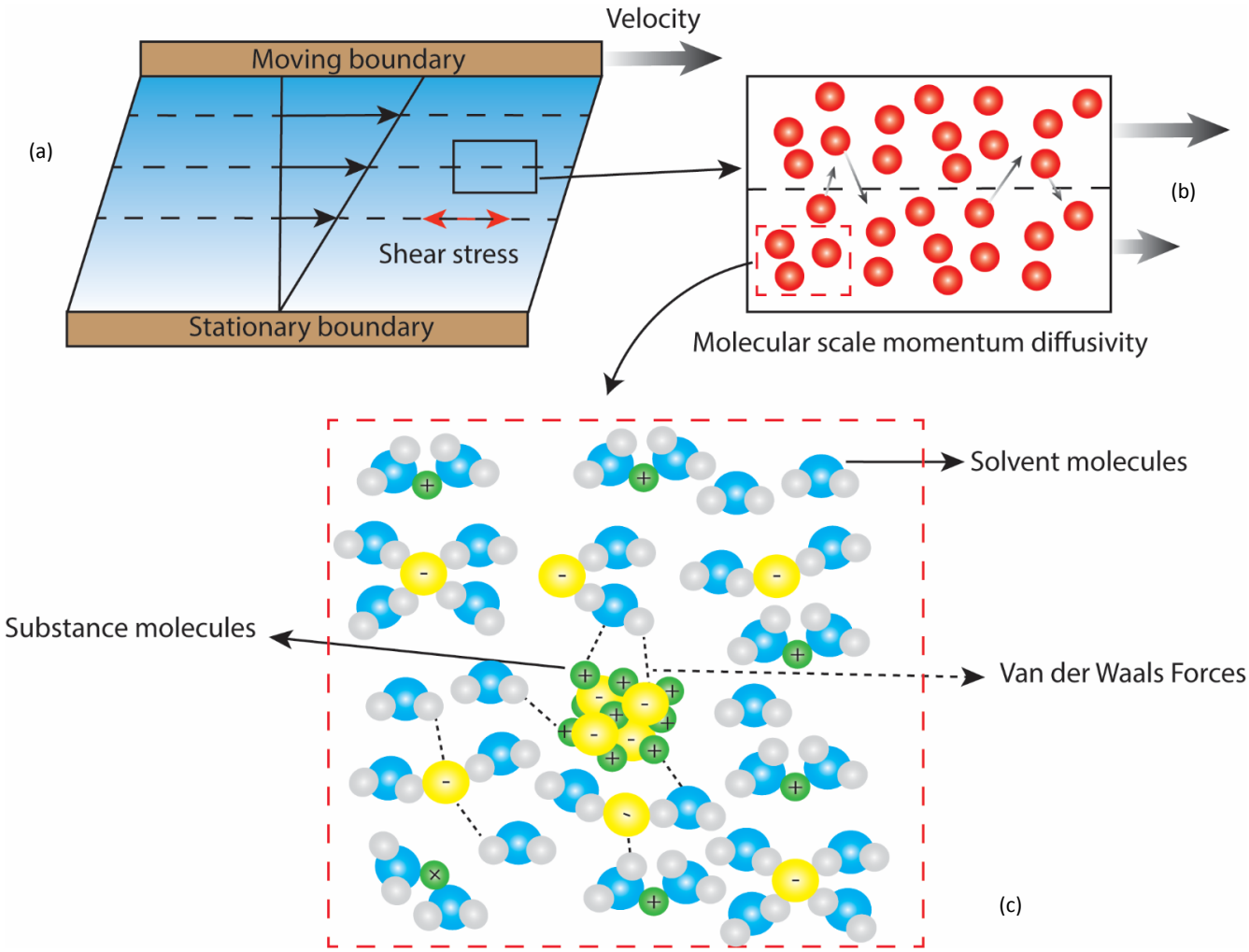


Figure 4-1- a) schematic view of fluid layers sliding on each other, b) interlayer molecule transfers c) atomic scale forces

To measure fluid parameters, there has been lots of interest towards employing microelectromechanics (MEMS) based viscometers due to their accuracy, simplicity and compatibility with different industries. There are three types of microsensors that have been used mostly in dynamic viscosity measurement which are vibrating microcantilever [70] and plate, quartz crystal resonators [71] and microfluidic systems [72]. The governing equations of these micro-viscometers are as follows.

Vibrating microcantilever [70]	$\frac{\mu}{\rho} = \frac{\pi b^2 \Gamma_r(\omega_R)}{4((\omega_{vac}/\omega_R)^2 - 1)}$ Eq. (4.1)
Quartz crystal resonator [71]	$\mu\rho = \frac{\pi E_Q \rho_Q (\Delta f)^2}{f_o^3}$ Eq. (4.2)
Microfluidic sensors	Transduction principles are: Pressure difference, image processing, impedance variation

Where μ is fluid dynamic viscosity, ρ is fluid density, ω_{vac} is frequency response in vacuum, ω_R is frequency in fluid medium, b is microcantilever width and Γ_r is hydrodynamic function in the governing function for microcantilevers. In quartz crystal resonators, E_Q is elastic modulus, ρ_Q is density of the quartz crystal, Δf and f_o are frequency shift and natural frequency of free crystal, respectively.

Quartz resonators employ piezoelectric surfaces which can make high frequency vibrations with low amplitude. The measured resonance feedback is proportional to fluid density and viscosity that is modified when a change happens in shear stress of fluid [73, 74, 75, 76]. This method has been successfully employed in industrial real-time detection applications [77, 78] even though some ranges of fluids viscosity -specifically in comparative applications- are not detectable due to low amplitude vibrations of this measurement system [79, 80]. In biological application, micromachined Quartz resonators have been used as density and viscosity measurements systems [81, 82]. However, the piezoelectric films are not easily integrable during microfabrication [83].

Resonating cantilevers and plates have been the most frequently used type of MEMS viscometers [84] which are usually actuated by means of piezoelectric material [85, 86, 87],

magnetic field [88, 89, 90] or alternating electricity current [91, 92, 93]. Theoretical studies and models connect variation in fluid density and viscosity to shift in resonant frequency and quality factor, respectively [94, 95, 96]. In order to get a more sensitive microcantilever or plate resonator, a thin neck that connects microdevice to its base is required. However, the weaker the base, brings less reliability for the whole device as it will not endure for high cycles due to fatigue and stress concentration effects.

There are not many viscometers designed based on only fluid flow in a microfluidic channel. As an instance, integration of two electrodes in a microchannel and monitoring the changes in fluid impedance was used for viscometric study of blood [97]. With considering the non-Newtonian effect of fluids, some MEMS based microplate oscillating systems have been designed and the governing equations were derived based on Maxwell equations [92] as,

Vibrating microplate [92] $$\mu\rho = \frac{2}{\omega}\left(\frac{P}{aBU_o^2}\right)^2 \qquad \text{Eq. (4.3)}$$

Where, ω is oscillation frequency, U_0 is motion amplitude, B and a are plate width and length, μ is fluid dynamic viscosity, ρ is fluid density and P is average oscillation power over a period.

As it is apparent from the above mentioned formulations (Eq.4.1-Eq4.3), frequency change in microsystems submerged in fluid is dependent on both density and viscosity (μ and ρ). In other words, to extract viscosity using these microsystems, a knowledge of fluid density is required. Otherwise, in some articles, another parameter such as quality factor is measured to have another equation for the two unknowns.

Although these methods are addressing the viscosity measurement problem, these formulas are mainly useful for Newtonian and single-phase fluids where the viscosity changes can be assumed negligible with shear stress and density can be measured with other methods. However, in non-Newtonian or multi-phase fluids, both density and viscosity are subject to change at different flow rates since particles are getting segregated at different shear rates. Thus, the fluid is defined with its molecular organization which makes these two parameters namely, dynamic viscosity and density, interconnected. To study and measure fluid

properties, kinematic viscosity captures this interconnectivity of fluid properties at different flow conditions in addition to being measured easily by the proposed platform.

Kinematic viscosity measurement is most frequently used in chemical processing and oil industries where fluid is sheared and displaced at the same time. On the other hand, processing chemicals such as crude oil is happening during a long process and in each step a certain fluid with some concentration is getting separated/added and the rest will go to the next processing machine. During each process it is very difficult to calculate and measure fluid dynamic viscosity or density as the concentration of each parameter should be precisely determined while the process is ongoing quickly. Thus, kinematic viscosity can be used as a single parameter in this industry to qualify the chemicals specification at a certain processing step. The effect of this addition at each step on the final fluid viscosity is called intrinsic viscosity or viscosity intensifying effect which is calculated using following formula [98]:

$$[\mu] = \lim_{c \to 0} \frac{\mu_r - 1}{c} = \lim_{c \to 0} \frac{\mu_{sp}}{c}$$

$$\mu_{sp} = \frac{\mu - \mu_0}{\mu_0} = \mu_r - 1$$

Eq. (4.4)

Where μ is solution viscosity, $[\mu]$, intrinsic viscosity

μ_r, relative viscosity, μ_0, initial viscosity

μ_{sp}, specific viscosity

To obtain kinematic viscosity from dynamic viscosity, measuring of solution density which changes with different concentration (c) is required. Here, to get rid of this density measurement, the fluid specifications will be identified using kinematic viscosity which can be measured as a solution parameter using devices such as capillary viscometers. However, it is shown that kinematic and dynamic viscosities are substitutable in some conditions based on value of intrinsic kinematic viscosity. In order to justify this need and replicability of measuring kinematic viscosity instead of dynamic viscosity, for example material B which is solvable in solution or fluid A is considered. To obtain intrinsic kinematic viscosity, $[\upsilon]$, and its relation with intrinsic dynamic viscosity following formulation has been derived [99].

$[\upsilon] = \lim_{c\to 0} \frac{\upsilon_r - 1}{c} = \frac{1}{\upsilon_0} \lim_{c\to 0} \frac{d\upsilon}{dc}$ $\mu = \rho\upsilon$ $\frac{d\mu}{dc} = \rho \frac{d\upsilon}{dc} + \upsilon \frac{d\rho}{dc}$ $[\mu] = [\upsilon] + \frac{1}{\rho_0} \lim_{c\to 0} \frac{d\rho}{dc}$	Where $\rho = \frac{g_A + g_B}{V}$, $V = g_A V_A + g_B V_B$ $c = \frac{g_B}{V}$ thus, $\frac{1}{\rho_0} \frac{d\rho}{dc} = \frac{1 - V_B \rho_0}{\rho_0}$	Eq. (4.5)

Where υ_0 and ρ_0 are the kinematic viscosity and density before change, υ_r, is relative kinematic viscosity, V is solution volume, g_A and g_B are mass of each compartment and c is concentration amount. As it is shown in the formulation, in some solutions where $\frac{d\rho}{dc}$ is negligible, intrinsic kinematic viscosity and intrinsic dynamic viscosity are replaceable and kinematic viscosity can be used equally as dynamic viscosity to specify the solution in each processing step. This condition is applicable in oil industry and for some range polymers where $\frac{d\rho}{dc}$ is in the order of one ten-thousandth (0.0001) and intrinsic viscosity value is large. In protein solutions $\frac{d\rho}{dc}$ is in the order of one thousandth and intrinsic viscosity value is very small and in the order of one hundredth [99]. However, still kinematic viscosity measurement can be used to avoid dealing with determining partial concentrations of solution. In all of the abovementioned measurement systems, viscosity detection is linked to density. Though these two parameters have different physical meaning, interpreting these parameters together will lead us to a unique parameter as kinematic viscosity to understand and detect changes in fluid properties.

Kinematic viscosity can be interpreted as momentum diffusivity between adjacent fluid layers. In other words, momentum flux between fluid layers is proportional to gradient of mass flux there. This momentum diffusivity is related to density and viscosity of fluid which in total is named kinematic viscosity. The following formulation shows this concept better.

F= m a = m v/t = momentum/time and $\tau = \mu \frac{dv}{dy}$ Eq. (4.6)

The Newton formula can be written as momentum over time and force per unit area is stress. Thus, rate of momentum per unit area is equal to viscosity (μ) by velocity gradient between layers. On the other hand, if we consider non-Newtonian fluids, the formula is re-written as follows:

$$\tau = \frac{\mu}{\rho}\frac{\rho dv}{dy} = \upsilon\frac{d(\rho v)}{dy}$$ Where ρV is mass flux between layers Eq. (4.7)

Thus kinematic viscosity represents momentum transport per unit area between layers. Figure 4-1 shows a fluid between to plates which one of them moves and drags the fluid. This will create different fluid layers which slide on each other. However, molecules are getting transported between two adjacent layers due to the gradient of flow velocity between these two layers. In other words, the faster layer is pushing the slower one to move faster and the slower layer does vice versa. This will create a flux of molecules between layers which is dependent on the intermolecular Van der Waals forces and molecular mass. The combination of these two parameters, the resistance force and inertia, is kinematic viscosity.

In other words, kinematic viscosity determines how fast a fluid flows when a certain force (shear) is applied on it. Though, dynamic viscosity mainly considers the inter-molecular forces which determine the friction force that stops or slows a fluid from flowing.

4.2. 3D suspended polymeric microfluidics

All of the applied micro-viscometers work based on moving a microstructure inside a fluidic medium. Since this motion replaces a fluid mass while shears it, the density effect and consequently density measurement is inevitable in this kind of measurement systems. On the other hand, any variation in solution concentration will change both density and viscosity. Thus, in order to specify a fluid under different processes, a kinematic viscosity measurement system is introduced in this study. The novel microsystem is designed based on the capillary kinematic viscosity measurement concept [100] which is depicted simply in Figure 4-2.

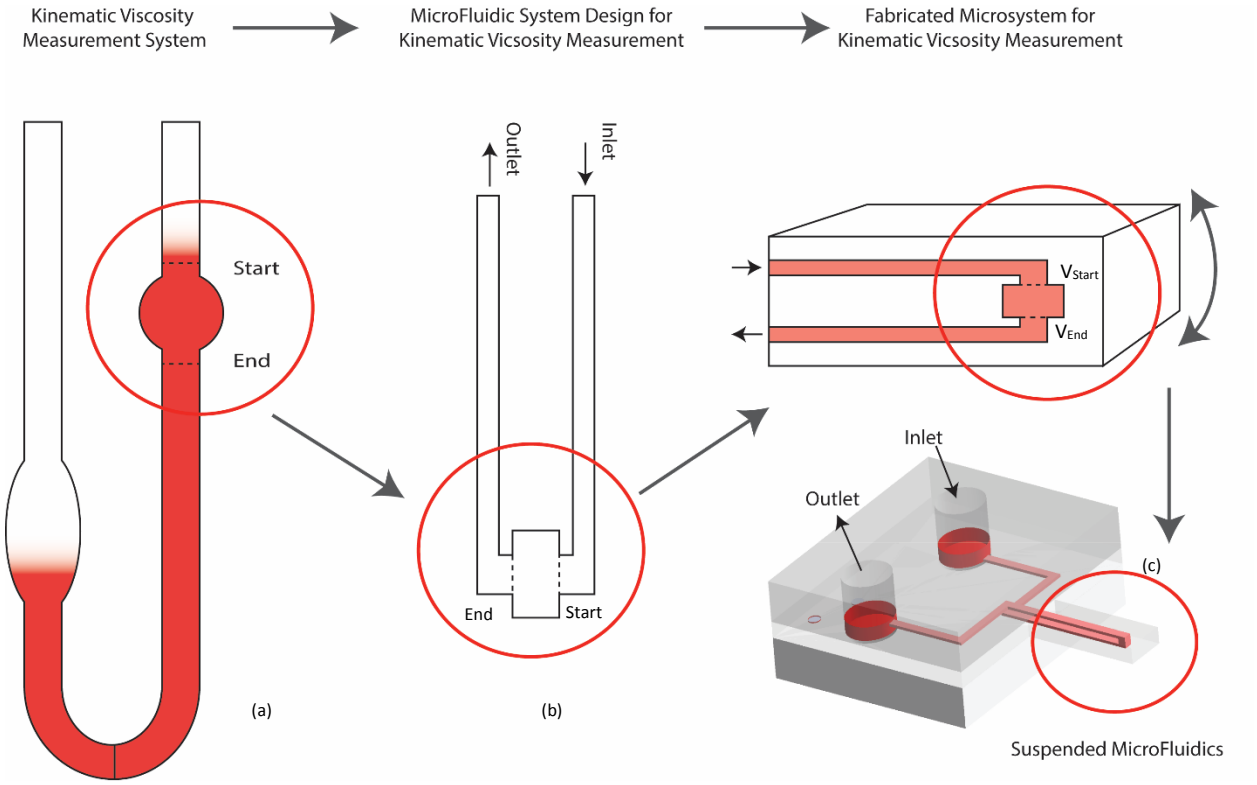


Figure 4-2- a) Capillary kinematic viscosity measurement system, b) Microfluidic system designed based on capillary system, c) Suspended microchannel designed to transduce and measure fluid forces

The old system works based on free fall of fluid due to its mass which creates a constant pressure difference at two sides of the target zone. Writing a force balance equation between two graduation marks namely, start and end, on viscometer will result in fluid kinematic viscosity measurement. Since the only acting forces are fluid shear force and weight force in a constant volume between the marks, measuring time when fluid falls between these two marks on viscometer is proportional to fluid kinematic viscosity as shown in the following formulation [101].

$$\mu = \frac{A(\rho g h)a^2}{8lQ} \quad \text{or} \quad \upsilon = K\,t\ , \qquad K=\pi g h a^4/(8lV) \qquad \text{Eq. (4.8)}$$

Where a is the capillary radius, V is fluid voume between marks, l is distance of two measurement points, Q is flow rate, g is acceleration due to gravity, μ is dynamic viscosity, υ is kinematic viscosity, ρ is density, t is time and h is pressure difference height.

The 3D suspended microfluidic system was designed in previous chapter to detect the flow and its properties such as density and viscosity. After considering the viscometers and flowmeters mentioned in the introduction section, a microcantilever with embedded microchannel design was noticed that had a very interesting design to detect fluid flow even though this capability had not been considered by its initiators. This microsystem [52, 49] was comprised of a U-shape microchannel inside a microcantilever (Figure 4-3a). The suspended microchannel was used to detect microparticles and cells using either frequency shift or deflection measurement method. However, another interesting aspect of this embedded microfluidics is flow forces applied to the microcantilever. The change in flow direction creates momentum force that will be modified when fluid properties such as kinematic viscosity vary. Detecting these forces can be an innovative way to study flow properties in a dry and closed-loop system.

Since the microchannel plane is parallel to the microcantilever plane, the applied flow forces were not strong enough to bend the microcantilever in this direction (Figure 4-3a). Hence, these systems were microstructures for only frequency measurements. But, in this study, this issue is resolved by designing the microchannel plane to be orthogonal to the neutral or bending plane (Figure 4-3b). This will increase the system sensitivity up to 5 times more than the earlier designs. This new sensitivity value is due to change of flow force direction to the least stiff plane of the microcantilever.

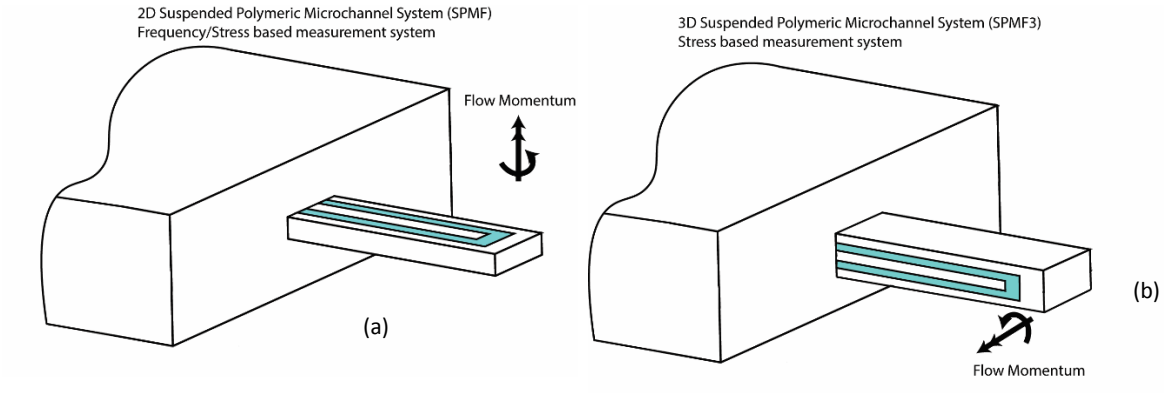


Figure 4-3- The design comparison between 3D suspended microfluidic system (a) and 2D suspended polymeric microfluidics (b)

The novel suspended microsystem works based on the constant flow rate inside a microfluidic channel which creates a constant pressure difference and velocity difference at two sides of the aperture. This makes flow forces which are applied to the microcantilever. The designed microchannel is embedded inside a microcantilever to employ the flow forces, applied to the microchannel walls. These flow forces deflect microcantilever depending on changes in flow properties as the flow rate is kept constant.

Writing force balance equation for the microfluidic system considering the equations of the old capillary measurement system (Eq. 4.9) results in an equation for kinematic viscosity measurement using the SPMF^3 (Eq. 4.10). Following are the equations of both systems and their relation to kinematic viscosity. Replacing Δp in old capillary system equation, (Eq. 4.9), with the Δp from suspended microfluidics force equation, f, results in the kinematic viscosity measurement equation (Eq. 4.10).

Capillary viscosity measurement system [101]	Suspended microfluidics
$\mu = \frac{A\Delta P a^2}{8lQ}$	$f = \dot{m}\Delta V + A\Delta p = k_m \delta,$
	$k_m \cong \frac{3EI}{L^3}$ Q=$\dot{m}/\rho$

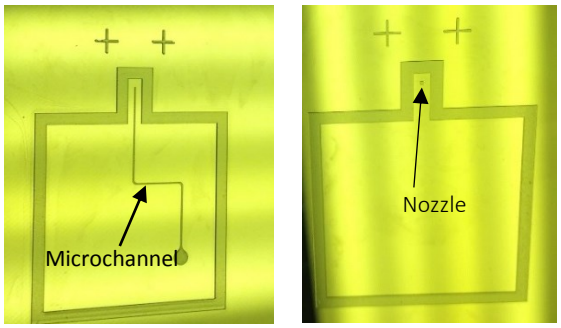
Q= Volume/time Eq. (4.9)	$\frac{k_m \delta a^2 - \dot{m} \Delta V a^2}{8 l \dot{m}} = \upsilon$ Eq. (4.10)

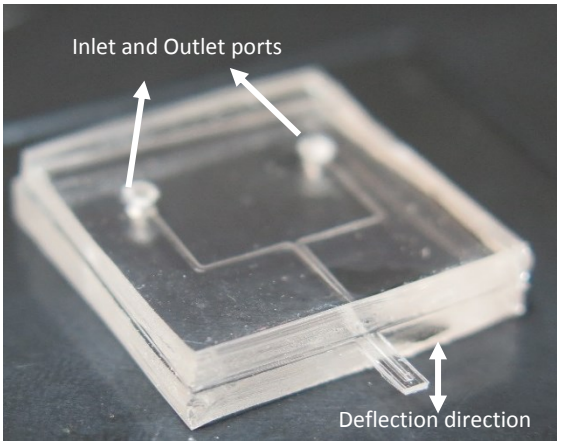
Where, A is cross section area, l is the nozzle length between start and end marks, Δp and ΔV are pressure and velocity difference between two graduation marks, k_m is cantilever stiffness, $\dot{m}$ is mass flow rate and δ is cantilever deflection. If the volume between the two marks is considered as a control volume, f shows the applied due to pressure and velocity difference between two ends of this volume. These differences are created as a result of changes in flow direction and channel cross section area. The applied force is equal to the cantilever spring force, $k_m \delta$. The above mentioned formula, Eq. 4.10, shows a linear relationship between the microcantilever deflection and kinematic viscosity of the fluid which is brought here after a simplification step (Eq. 4.11).

$$\upsilon = k\delta - S \qquad \text{where } k = \frac{k_m a^2}{8 l \dot{m}} \;, S = -\frac{\Delta V a^2}{8 l} \qquad \text{Eq. (4.11)}$$

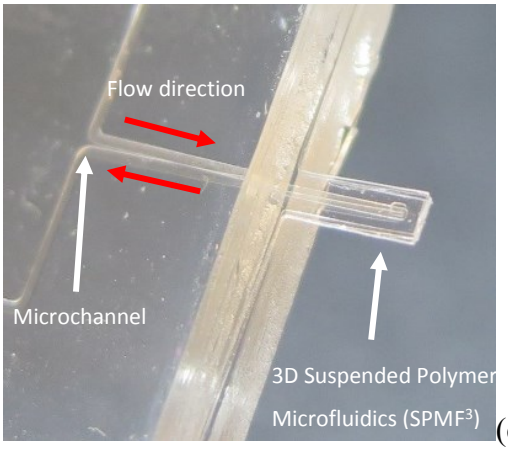
4.3. Fabrication of the device

In order to fabricate the 3D microchannel inside a microcantilever structure, three different layers were designed and bonded together to create the whole suspended microfluidics. These three layers consist of two microchannel layers and one nozzle layer. As it is shown in Figure 4-4, two different molds, one for microchannel layers and one for nozzle layer, are fabricated using soft lithography method. According to the designed microcantilever thickness, SU8-2075 was selected as a photoresist in the mold fabrication process. Then, two microchannel layers were fabricated using the microchannel mold and bonded to a nozzle layer in between to form the 3D suspended microfluidic system. The bonding process is done using plasma activated technique followed by microchannel and nozzle layers alignment under a wide-range microscope. Failure in bonding strength and alignment will result in misalignment between layers. These microchannel and nozzle layers are made of Polydimethylsiloxane (PDMS) which also increases the amount of deflection or sensitivity to small loads due to low elastic modulus. The detailed view of microcantilever tip shows the embedded microchannel, flow and deflection directions (Figure 4-4c).





(b)



(c)

Figure 4-4- a) Microchannel and nozzle molds for PDMS microfabrication, b) Fabricated 3D suspended polymeric microfluidic system c) Detailed view of the SPMF^3

4.4. Prediction and experimental validation

In this section, an experiment is performed to measure changes in fluid kinematic viscosity using the 3D polymeric suspended microfluidics. To modify kinematic viscosity of fluids under experiment, DI water with different concentrations of salt, 0-15% as a solution, and milk with fat concentrations of 0-35% as a colloidal fluid have been considered. An optical laser deflection measurement system has been used to detect microcantilever deflections against variations in fluid properties (Figure 4-5).

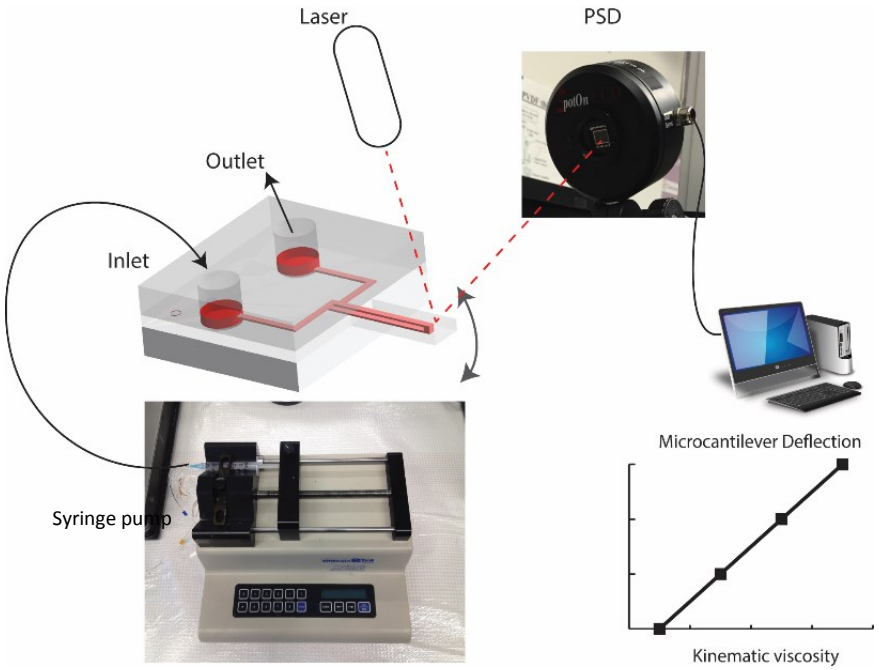


Figure 4-5- Laser displacement measurement system

According to the salt solutions in Table 4-1, kinematic viscosity variations through the addition of salt can be detected using the SPMF[3]. However, both density and viscosity increase when salt is added to DI water. As such, Table 4-2 summarizes the microcantilever deflections against changes in fat content of milk. Milk viscosity increases with fat concentration while its density decreases [102]. Thus, the source of this deflection can be due to either density or dynamic viscosity or both.

Table 4-1. Changes in DI water and salt solution properties and experimental deflection results; both density and viscosity increases with salt concentration

Salt wt %	Density (kg/m^3)	Dynamic Viscosity (cP)	Kinematic Viscosity (cSt)	Deflection (µm)
0	999	1.002	1.00	2.51
10	1070	1.193	1.11	2.75
15	1110	1.350	1.21	3.08

Table 4-2. Changes in milk properties with different fat content and experimental deflection results; density and viscosity change in opposite way

Fat wt %	Density (kg/m^3)	Dynamic Viscosity (cP)	Kinematic Viscosity (cSt)	Deflection (μm)
0	1033	3.594	3.48	10.48
3.25	1030	4.192	4.07	11.90
10	1025	4.797	4.68	14.85
20	1012	6.598	6.52	20.80
35	994	11.391	11.46	36.21

To further investigate the source of this change a finite element analysis (FEA) has been done with different fluid densities while the viscosity is kept identical and vice versa. In this simulation, a microcantilever with a dimension of 6000×2000×600 μm, length, width and thickness respectively, with an embedded microchannel of 200×100 μm was modeled. The flow rate was kept constant as 50μl/min and the fluid was water. This analysis has been done using two modules of CFX and structural to solve Navier-Stockes equations of steady state fluid dynamics and structural behaviors to predict the resultant deflections due to applied flow forces on the microcantilever in ANSYS.

As given in Table 4-3, the predicted variations of deflection show that microcantilever deflection decreases when density is increased at constant viscosity while its deflection increases when viscosity is increased at constant density.

Table 4-3. Fluid density and viscosity inputs and predicted cantilever deflections

Density (kg/m^3)	Viscosity (cP)	Deflection (μm)
997.8	1.007	2.44
1073.3	1.007	2.29
1123.6	1.007	2.20
997.8	1.184	2.83
997.8	1.361	3.29

Since the flow rate is kept constant during experiments and prediction, increasing of density will decrease the pressure difference leading to lower velocity difference and hence the deflection. On the other hand, when viscosity is increased, pressure difference increases which result in higher microsystem deflection (Figure 4-6). Therefore, the FEA modeling also confirms that both density and viscosity variations affect the SPMF[3] deflections similar

to the experimental results. However, the FEA results have confirmed that the viscosity and density effects are opposite to each other.

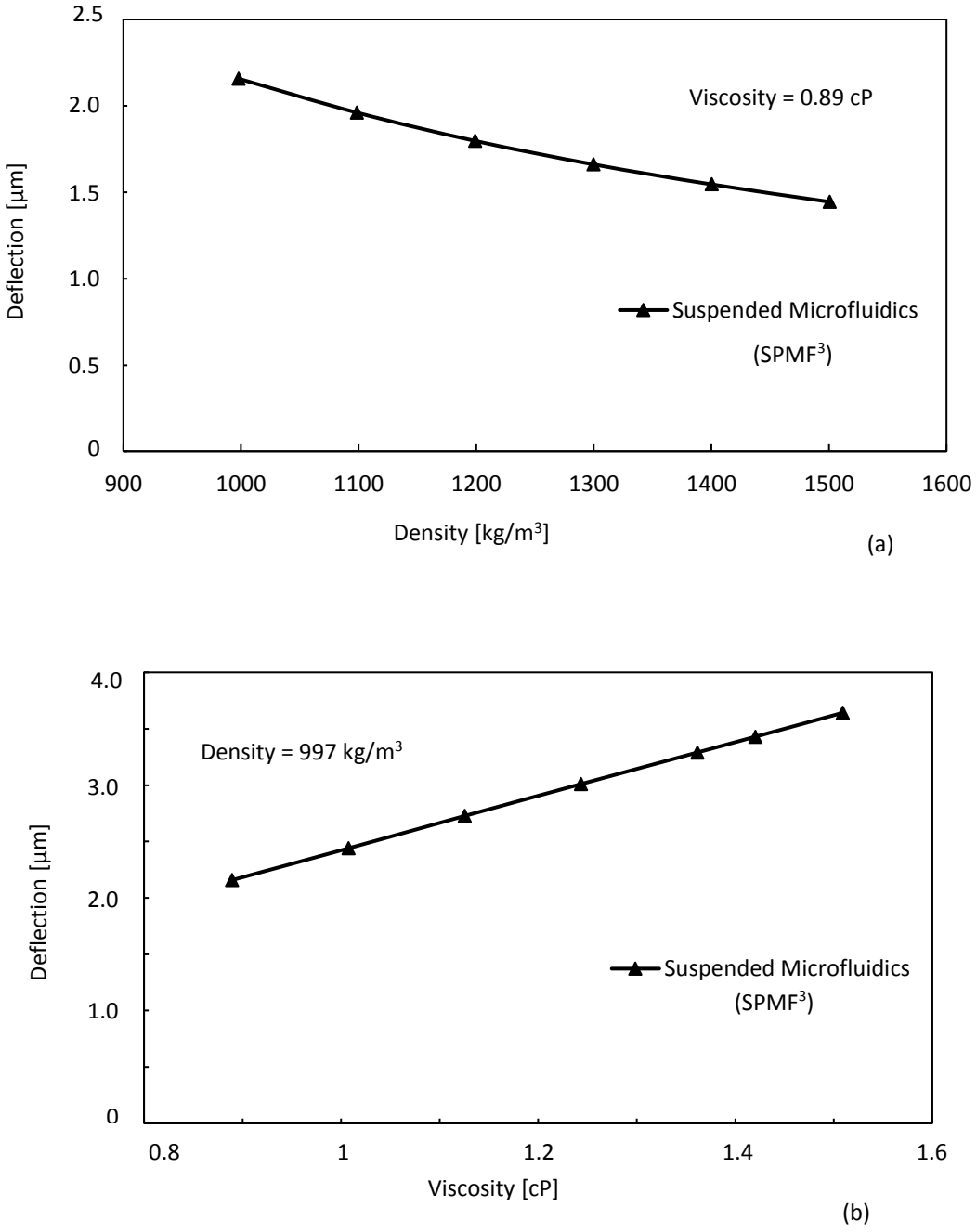


Figure 4-6- Finite element analysis results of microcantilever deflection when fluid density or viscosity is changed, a) against density at constant viscosity, b) against viscosity at constant density

As explained earlier, viscosity and density are coupled in influencing the structural behavior and a comprehensive parameter would be helpful to capture their influence on microcantilever deflection. Hence, kinematic viscosity which considers the combined effects of dynamic viscosity and density is used to represent the fluid to capture the variation of dynamic viscosity and density.

To address this issue here, the simulation results are presented with kinematic viscosity (Figure 4-7) over a wide range which show that how these two parameters, viscosity and density, can specify the fluid under study when coupled together as a single parameter. The kinematic viscosity range is chosen to represent simple Newtonian fluids such as water to complete non-Newtonian fluids such as milk and blood. Using design parameters of the SPMF^3 in Table 4-4, the theoretical formula prediction (Eq. 4.11) is with FEA results. The derived theoretical formula is simplified as $\upsilon = k\delta - S$ where, k is the resultant cantilever stiffness. The theoretical formulation can be used to design any SPMF^3 for required kinematic viscosity measurements.

Table 4-4. Suspended microfluidics design parameters

Microchannel hydraulic diameter, a (μm)	133
Velocity difference at nozzle sides, ΔV (m/s)	0.002
Microcantilever stiffness, k_m (N/m)	0.035
Mass flow rate, $\dot{m}$ (kg/s)	8.33×10^{-7}
Nozzle length, l (μm)	300

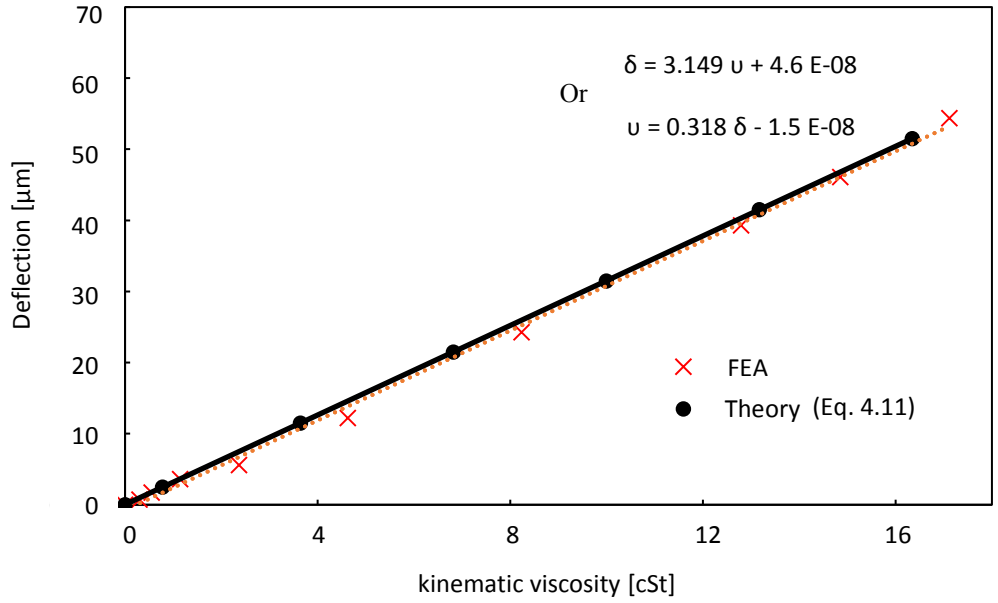


Figure 4-7. FEA and theoretical results of microcantilever deflection against kinematic viscosity

In other words, any variation in fluid concentration cannot exactly be predicted with only density or viscosity since both are changing and each parameter has a different effect on the suspended microfluidics. The proposed suspended microcantilever has a unique response to changes in kinematic viscosity as it is shown here validating the SPMF^3 as an appropriate tool for measuring fluid property with kinematic viscosity.

In a similar way, prediction has been continued for other fluids such as acetone, DI water, blood, milk and the results were presented against kinematic viscosity in Figure 4-8. These fluids were selected to basically cover a reasonable range of the fluids covering Newtonian and non-Newtonian fluids together. In order to validate the SPMF^3 platform for kinematic viscosity measurements, experiments with water, salt solution and milk, were carried out and the results are compared with finite element analysis and simplified prediction formulation in Figure 4-8. As it is shown here, there is a good agreement among the theoretical, finite element and experimental results. These results were shown in the logarithmic format in order to represent fluid properties in equally expanded regions. As it is shown, kinematic viscosity can be employed for studying variation in fluid properties specifically in a more comprehensive way.

The error bars of experimental results were calculated according to the geometrical scheme of laser deflection measurement system. There are three main sources of error such as laser angle, PSD angle and microcantilever position that should be considered in this experiment. According to the geometrical calculations [103], variation in each of the abovementioned parameters may add an error on the final microcantilever deflection up to 10% of the measured value.

In the former studies, fluids are mostly considered Newtonian however, the interpretation of microcantilever deflection is difficult if it comes to an unknown fluid where the changes in dynamic viscosity and density are unknown. Moreover, if the fluid is non-Newtonian or with multi-phases, interpretation of experimental results and changes in microsystem deflection will be unclear. This, as well as the formulation in the previous section, clearly states how viscosity and density of fluids are related when it comes to detecting changes in a fluid through an external force or effect. Thus, the new SPMF^3 which is designed for kinematic viscosity measurement addresses the interconnectivity of density and viscosity.

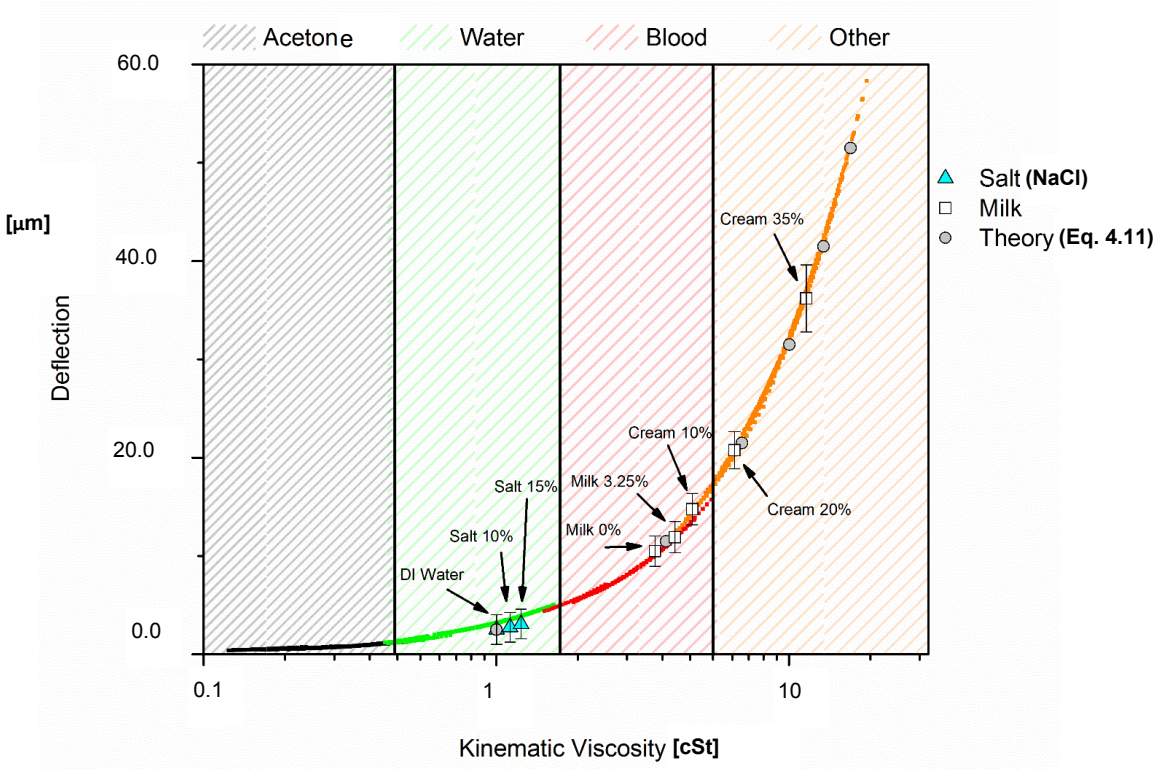


Figure 4-8. Prediction and experiment comparison of deflection behavior for various fluids

4.5. Conclusions

In this study, a practical use of kinematic viscosity is given as a comprehensive fluid parameter. In order to measure fluid parameters and avoid former viscometer issues such as the necessity of density value during viscosity measurement, a 3D suspended microfluidics has been designed based on capillary measurement system. Detailed FEA modeling of the $SPMF^3$ shows different behaviors of microcantilever against fluid density and dynamic viscosity variations. Using kinematic viscosity as a comprehensive parameter, both Newtonian and non-Newtonian fluids were studied and tested in a less complicated manner.

In order to validate the kinematic viscosity effects and importance, the 3D suspended polymeric microfluidics was developed along with theoretical and finite element analysis. Solutions of DI water and salt of different concentrations and milk with a variety of fat contents were injected to the fabricated microchannel and the microcantilever deflections were measured according to variations of solution concentration. Then, the $SPMF^3$ deflections against kinematic viscosity variations were studied.

Finally, with a comparison between theoretical and experimental results, it was confirmed that the kinematic viscosity is a unique parameter in fluids that can capture the influence of fluid properties on structural behavior. Furthermore, the proposed $SPMF^3$ platform shows promising results as a microsystem for kinematic viscosity measurements covering with Newtonian and non-Newtonian fluids and biological and non-biological fluids, in a unique way.

Chapter 5

Rigid and elastic microparticles detection using 3D suspended polymeric microfluidics (SMPF^3) with flow orthogonal to bending (FOB) configuration

Microsystems have gathered great interests and shown valuable results in the study of mechanobiology and biophysical analysis of cells due to their properties such as: microsystems match cell dimensions, provide growth microenvironment such as in-vivo environment, facilitate parallel analysis that can be done on cells through integration of other sensors or chemicals at low cost and with low amount of sample use. There are a variety of microsystems that measure cell properties such as physical, mechanical and chemical. In this study, a new 3D suspended polymeric microfluidics (SPMF^3) platform for microparticles detection is introduced and tested. The principle of the SPMF^3 is based on bending of structure due to flow forces applied to the microcantilever which is modified when microparticles are passing through the suspended microfluidics. The SPMF^3 is less complex and less expensive compared with the other optical, microfluidics and microcantilever based techniques formerly employed for microparticles and cells. According to the experimental results, the SPMF^3 is highly sensitive to the particles passing through the micro-nozzle without employing an external exciter. One can study and obtain the biophysical and elastic properties of the passing particles such as size, number and viscoelasticity with the deflection behavior of the microcantilever.

5.1. Introduction

One of the main microsystems which were employed to perform cell analysis is microcantilever [104]. The high amount of interest in microcantilever systems is due to their advantages in precise manipulation of cells or particles, determining the amount of applied force, quantification of cellular reactions and ability to measure any kind of force such as tensile, contractile and indentation. To this end, microbeads have been employed to mimic cells in biodiagnostic systems.

Microfluidic systems are the other main microsystem employed for biodiagnostic applications which came into effect much earlier than microcantilevers in the early 1970s [6]. These systems need an electrical or optical detection system in order to measure the bioparticles specifications inside microfluidic channels.

Microcantilevers have shown great promise in mechanobiological study of cells using which even particles weighing femtogram have been detected and studied [1]. However, delivering cells mixture to the microcantilever needs a fluidic medium which limits sensitivity of the microsystem. This low sensitivity is due to low quality factor of moving microstructures in fluidic medium since the liquid damping is much higher than damping in air and vacuum. Moreover, evaporation of fluid that happens in fluid surrounding microcantilevers affects the repeatability of their results. Craighead et al. [65] used this technique to detect single cell of E. Coli by measuring frequency changes of a microcantilever in vacuum condition instead of atmospheric pressure to increase sensitivity of their microsystem to 1.1 Hz/fg.

In order to improve the sensitivity and repeatability, suspended microchannel resonator (SMR) method has been introduced and tested [49]. This microsystem has been designed and fabricated in silicon chip which makes it very compact, with high sensitivity and repeatability. The only limitation with this silicon suspended microsystem is its high stiffness of silicon which limits its use in bending based measurement as it requires high force to bend. In addition, it requires external excitation when frequency change of suspended microchannel is monitored to extract biophysical properties of grabbed or flown bioparticles such as weight and count. To get rid of adding external excitation to SMR, the technique of making this microstructure out of a polymer such as Polydimethylsiloxane (PDMS) has been

introduced and tested. SadAbadi et al. [105] designed and tested this new suspended polymeric microfluidics (SPMF) which is less stiff than the silicon one so that bioparticles can deflect it with their physical properties such mass flow rate.

A 3D suspended polymeric microfluidics (SPMF^3) which is introduced in this study would increase the sensitivity of the suspended polymeric microfluidics from detecting bioparticles mass flow rate to a single microparticle detection. This microsystem detects microparticles through deflections of the suspended microchannel. Depending on the parameter under investigation, this deflection may be due to, shape, elasticity, weight or size of the microparticles. Moreover, the effect of particles flowing inside suspended microchannel will be intensified due to flow direction change and microchannel restrictions (the nozzle area) which result in more deflection and consequently higher sensitivity (Figure 5-1).

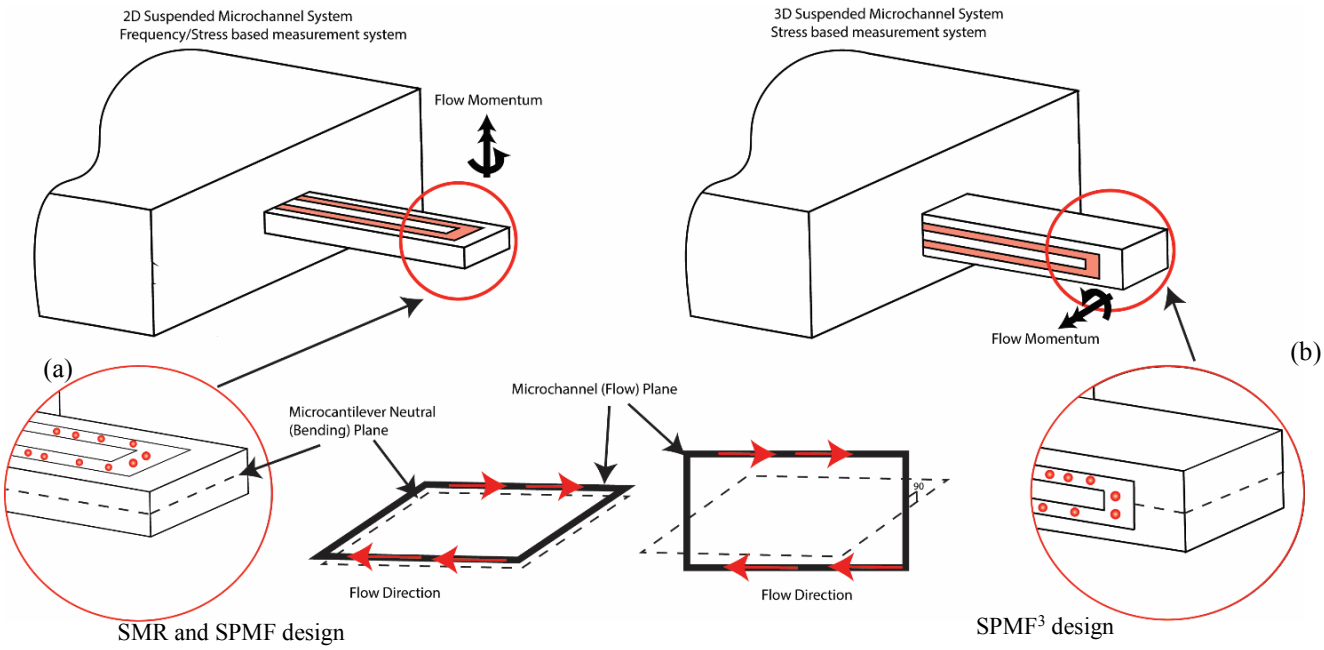


Figure 5-1. The suspended microfluidics design comparison a) 2D suspended microchannel resonator, b) 3D suspended microfluidics (SPMF^3); ▬ flow plane, ▪▪▪▪ neutral plane

For example, detection, counting and sorting of cells are done by current commercial cytometry machines. However, these cytometry devices are bulky and expensive. Besides, the experiment process needs high amount of sample, sample preparation and post processing times which require some specialists. This has motivated researchers' aim to integrate microsystem for biological and chemical processes towards developing less

expensive, portable and user-friendly microchips that are capable of implementing biological lab experiments [4]. Many transduction methods have been employed to detect variety of biomaterials using microsystems such as microcantilevers or microfluidics. In order to investigate cells using microsystems the most used methods are: Microfluidic resistive pulse technique, capacitance detectors, optical based systems, microcantilever resonator and microcantilever bending.

5.2. Microcantilever based detection

The capacity of microcantilevers in detection and measurements of biomolecular and cellular properties is apparent from the vast amount of investigations in this area [106, 107]. This detection is done using two techniques such as microcantilever deflection [108, 109] and frequency variation measurement [110, 111]. Deflection based detection method is mainly employed with polymeric microcantilevers or silicon based microcantilevers where bioelements with high mass flow rate or applied force is tested so that the applied deflection is measurable [112, 113, 114]. On the other hand, since the microcantilevers are mainly made out of silicon, frequency based method has more sensitivity in detection of smaller cells as the deflection is provided using an electric external excitation [115, 44, 45].

In microcantilever based detection, a fluidic medium is mainly employed to deliver the bioelement solution to the detection field which does not affect the sensitivity of the deflection based cantilevers though it deteriorates the functionality of frequency based systems [42]. The fluidic medium for microparticle delivery reduces quality factor of the microcantilever which affects its sensitivity. To maintain the high sensitivity of frequency based microcantilevers and overcome the shortcoming of surrounding fluid medium, two methods have been proposed in former studies. Replacing fluid with air [46] is one of these methods which resolves the issue to some extent however, the measurement precision is low due to contamination risk and fluid evaporation when the microcantilever is removed from liquid container and placed in measurement field. In another way, a microchannel is embedded inside a microcantilever to deliver particles to the detection zone [116]. This

microstructure which is called suspended microchannel resolved the issue of low quality factor and has increased the sensitivity of the microcantilevers.

When biomolecules are passing through the microchannel, these particles modify the resonance frequency of suspended microcantilever [117]. This frequency shift happens both when the bioelements are attached to the functionalized surface of the cantilever or just passed through the microchannel and are not trapped there. It has been shown that mass of single cells is measured through these two ways by monitoring variations in vibration frequency of the microcantilever. Having vacuum conditions around the suspended microchannel resonator (SMR), Burg et al. [49] measured particles mass of 1 fg (10^{-15}g) which was not accessible without increasing the sensitivity of this suspended microchannel and extending to a high quality factor of 15,000 which was as low as 1-10 in fluidic mediums.

Though the low throughput has been an issue in cytometric applications, sensitivity of detecting femto gram order bioelements such as E. Coli and Bacillus bacteria with high precision has been obtained by reducing the microfluidics flow rate and particles rate to 1-10 cells/second.

The SMR system was further tested for new applications such as particle volume and density measurement by Bryan et al. [50] in which a dual SMR system has been employed and cells were flown using two different fluidic solutions. Density difference between these solutions provides variations in buoyant mass measurements at each SMR which results in microparticles biophysical data such as volume and density. On the other hand, Lee et al. [51] replaced the laser with a piezoresistive bridge in order to have better resolution and packaging which ended in a single electrical package for this microsystems without laser deflection measurement devices.

5.3. 3D Suspended polymeric microfluidics (SPMF^3) for microparticle detection

In order to enhance the repeatability and sensitivity limits of the microcantilever based biodiagnostic systems, the suspended microchannel resonator solution made of silicon and integrated with electrostatic excitation was introduced [116]. This suspended microchannel concept was further improved to a 2D suspended polymeric microfluidics (SPMF) to detect

bioparticles without external excitation [105]. In these structures, flow plane was parallel to the bending (neutral) plane.

The direction change in fluid flow or dimension variation of the microfluidic channel, which creates flow forces, is an aspect of the 2D suspended microfluidics that can be used for bioparticle detection and analysis. Monitoring flow momentum forces had been one of the practical methods in commercial flow meters. Adding microparticles to the flow locally modifies the cross section area of microchannel which applies flow restriction force to the microcantilever as particles flow. However, in these two specimens of suspended microfluidics, SMR and SPMF, the microchannel is in the plane of microcantilever. In other words, the fluid flow and particle forces are applied to the stiffest direction of microcantilever and cannot deflect it.

In order to further improve the sensitivity of suspended microfluidics to fluid flow forces, a new concept of 3D suspended microfluidics is introduced which addresses the sensitivity issue by changing the microchannel plane to be perpendicular to the microcantilever plane. As it is shown in Figure 5-2, the SPMF^3 is comprised of a microcantilever in which two upper and lower microchannels as well as a middle nozzle are embedded. Since modification of fluid properties such density and viscosity affect flow properties such as velocity and pressure based on Navier-Stockes equations, any variation in fluid properties would be detected using the SPMF^3. Moreover, flow rate variations in a few microliter order can be easily detected due to high detectability of the microchannel plane modification. According to the simulation results, this innovative design has improved the suspended microfluidics sensitivity up to 5 times compared with its previous designs.

In order to fabricate the SPMF^3, three layers of two microchannels and a nozzle are bonded together. Soft lithography technique was employed for mold fabrication which was followed by the fabrication of two microchannel layers and a nozzle layer out of Polydimethylsiloxane (PDMS).

As it was mentioned earlier, the applied force is created due to flow forces and nozzle restriction. According to the simulation, theoretical and experimental results, if flow properties change or when microparticles are passing through the microchannel, pressure

and velocity values at two sides of the nozzle are modified which results in applied force variation to the microcantilever.

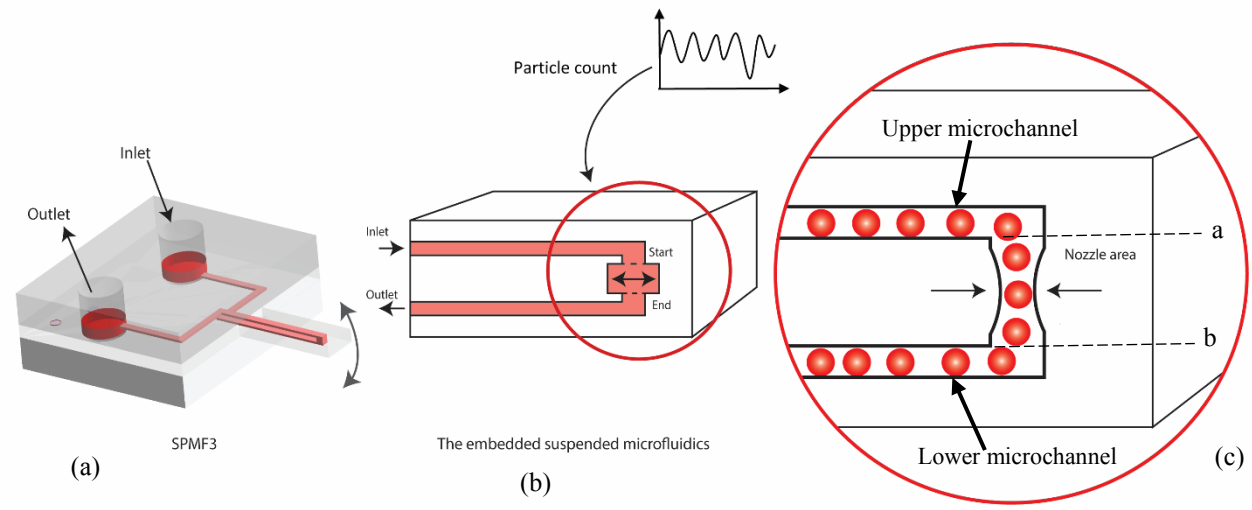


Figure 5-2. Microparticles detection using the 3D suspended microfluidics, a) The SPMF^3 platform, b) Detail view of the microchannel with variable nozzle area, c) Nozzle area restriction during particles flow

The governing equation of SPMF^3 is as follows: $f = \dot{m}\Delta V + A\Delta p = k_m\delta$, where Δp and ΔV are pressure and velocity difference between two sections of interest, namely 'a' and 'b', k_m is cantilever stiffness, $\dot{m}$ is mass flow rate, and δ is cantilever deflection. In a steady state flow, f shows the applied force to this field which is due to pressure and velocity difference between two selected sections across the nozzle. The applied force is equal to the cantilever spring force, $k_m\delta$.

This suspended microsystem detects, studies and counts microparticles through flow force as well as particles flow effects which apply steady or pulsating force on the microcantilever resulting in bending deflection.

5.4. Simulation and sensitivity study

In this section, applied forces to the microfluidics due to variations in microchannel dimensions are discussed and simulated. Changes in sizes of microchannel elements such as the micro-nozzle dimensions simulate the SPMF^3 behavior when a microparticle passes through the channel and restricts flow area temporarily. In order to study the microfluidics

behavior in response to nozzle dimensions, a finite element analysis (FEA) using ANSYS has been done in which microcantilever deflections were predicted for various nozzle cross section areas (Figure 5-3). In this modeling, first the fluid dynamic equations have been solved in CFX module and then the results were transported to structural module to predict the respective effects. Since the flow regime is steady during this analysis, the model was solved at steady state conditions. Thus, rectangle micro-nozzles with different cross section areas have been simulated and the results are depicted as follows.

Here, the nozzle widths, w, were set to 50μm, 300μm and 400μm and its length, d, was swept between 50-400 μm (Figure 5-4). This analysis was done with constant fluid properties such as density, viscosity and flow rate and the microcantilever sensitivity for different nozzle areas were predicted (Table 5-1). The microcantilever dimensions are 6000×2000×600 μm of length × width × thickness, respectively, with an embedded microchannel of 200×100 μm of width × depth, respectively.

Table 5-1. Fluid properties for finite element analysis

Flow rate (μl/s)	Viscosity (kg/ms)	Density (kg/m^3)	Fluid
50	0.9×10^{-3}	1×10^{3}	water

As it is shown in Figure 5-5, smaller the nozzle area higher the microcantilever deflection and sensitivity. The average fluid flow velocity is also shown versus the nozzle areas which also shows higher velocity at lower nozzle area or higher blockage (Figure 5-6a). Combining these two aforementioned results, the microcantilever deflection at different velocities due to nozzle blockage variation is shown in Figure 5-6b. In other words, blocking the microchannel temporarily or permanently produces a force variation which is detectable through the deflection of the suspended microfluidics. This blockage modeling simulates the real behavior of the $SPMF^3$ platform when microparticles are passing the microchannel and create transient flow restrictions.

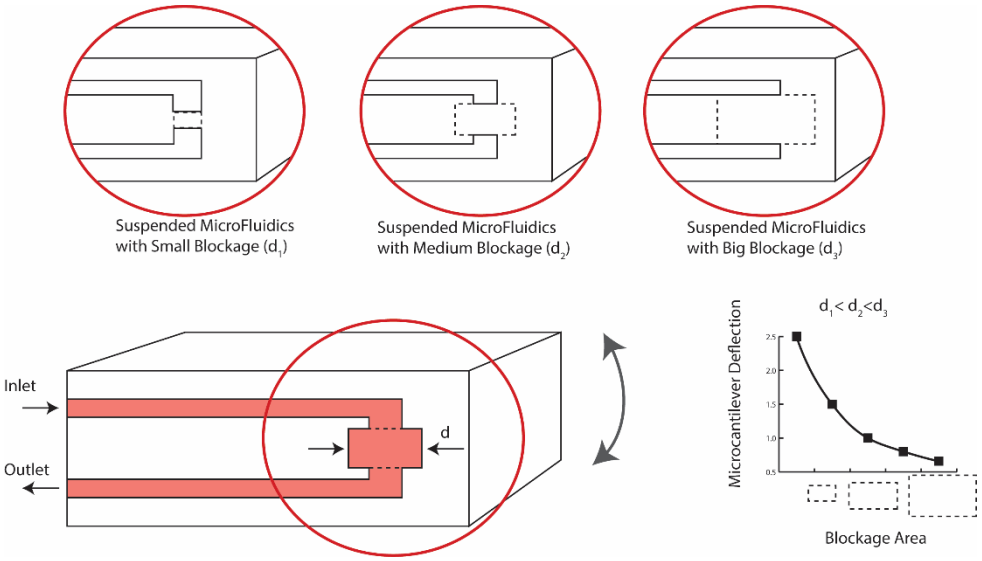


Figure 5-3. Scheme on effect of nozzle area on the microcantilever deflections

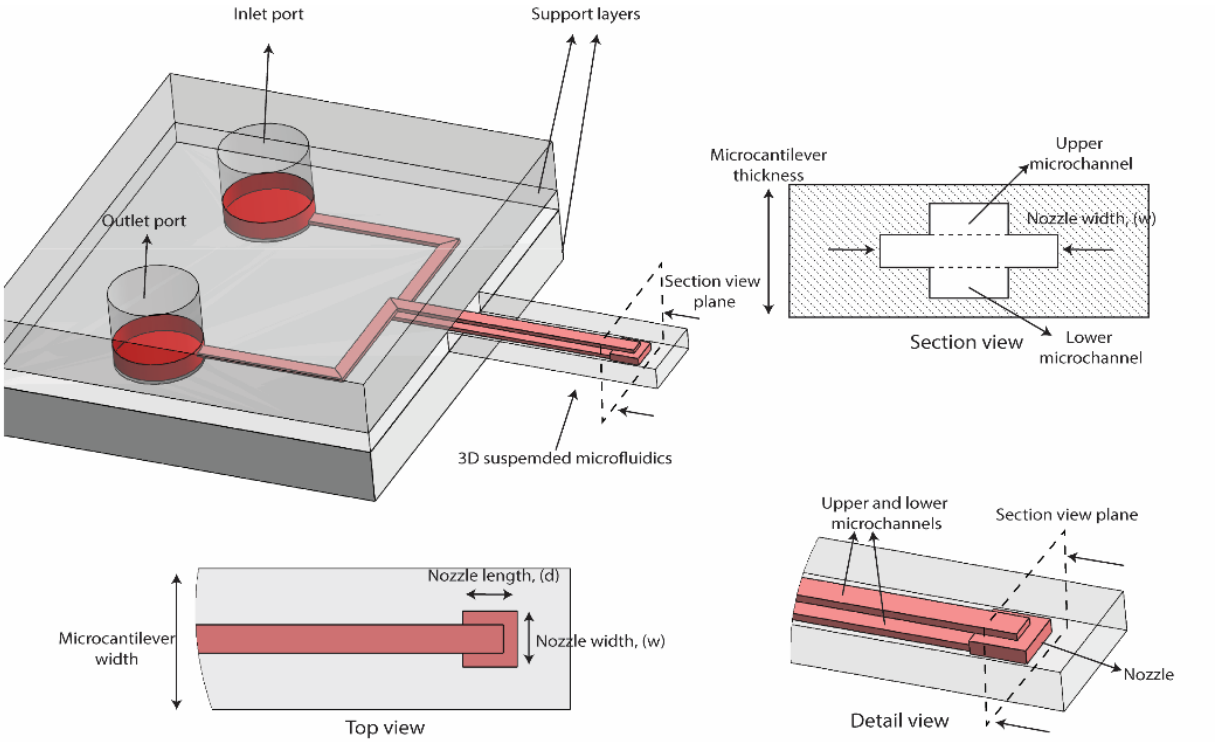


Figure 5-4. Detailed schematic views of the embedded micro-nozzle in SPMF[3]

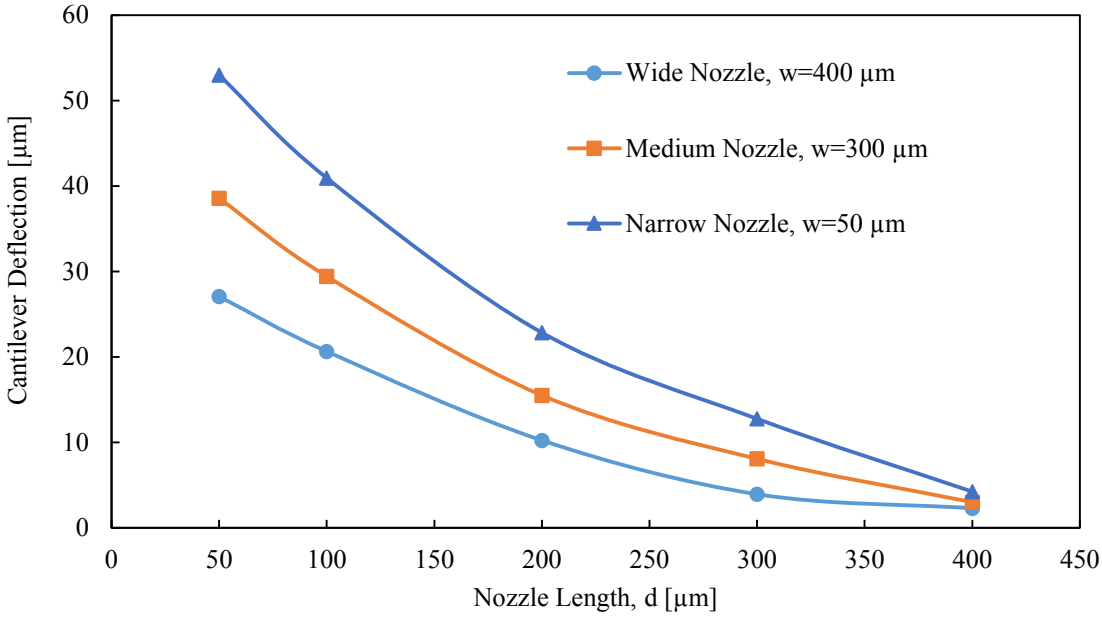


Figure 5-5. Effect of nozzle area (w×d) on the SPMF[3] behavior

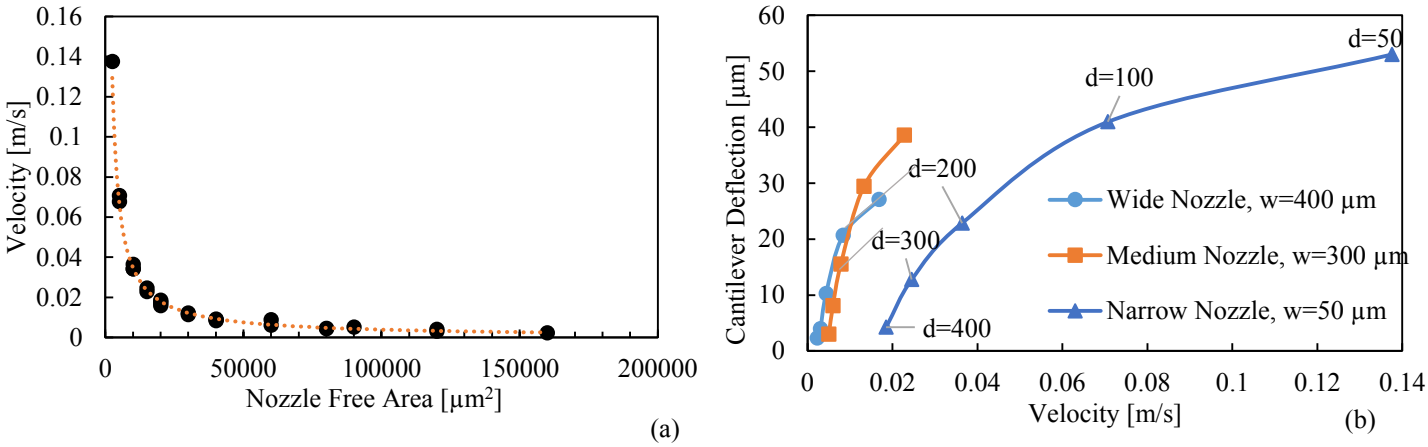


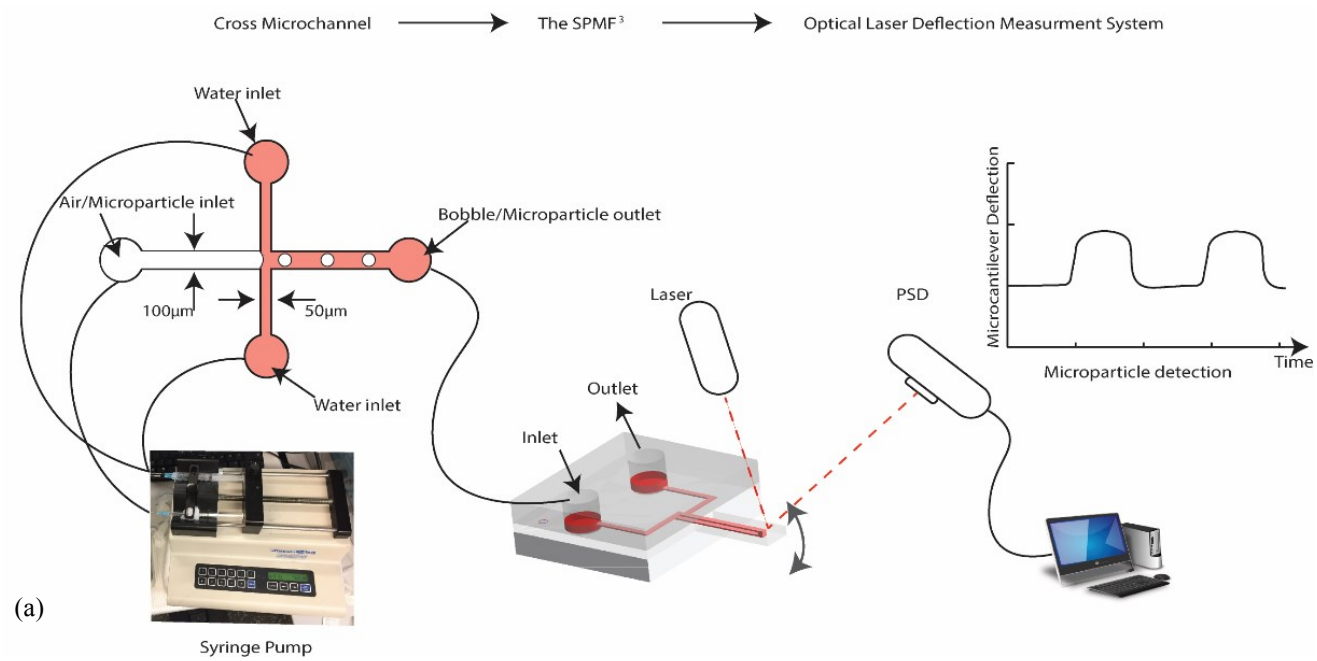
Figure 5-6. a) The flow velocity at nozzle versus variation of nozzle blockage areas, b) The resultant cantilever deflection for different flow velocities at various nozzle areas

5.5. Experimental validation

Rigid and elastic microparticles would be detectable when they pass through the suspended microfluidics. Here, polystyrene beads were employed as rigid particles and micro-bubbles

as elastic particles to validate the SPMF^3 performance. In order to test with micro-bubbles, two chips namely bubble chip and SPMF^3 were hybrid integrated. This compound test setup was designed to control microparticles flow rate inside the SPMF^3 and to avoid clogging of rigid particles inside the suspended microchannels. In bubble chip, the cross microchannel is made of two perpendicular microfluidic channels with dimensions of 100×100 µm of width × depth, respectively for the horizontal microchannel and 50×100 µm for the vertical one.

In order to generate micro-bubbles, two water inlets and one air inlet were employed with the cross microchannels. The bubble flow rate was controlled by adjusting air and water flow rates (Figure 5-7). Two water inlets and an air inlet are connected to syringe pump. Then, bubbles are flown to the SPMF^3 where the microcantilever deflections are monitored by an optical laser based deflection measurement system. Similarly, the rigid microparticles are injected inside the SPMF^3 through the cross microchannel. During the microparticles experiment, water inlets of the cross microchannel act as a sheath flow which help to direct microparticles and prevents their clogging at the channel walls.



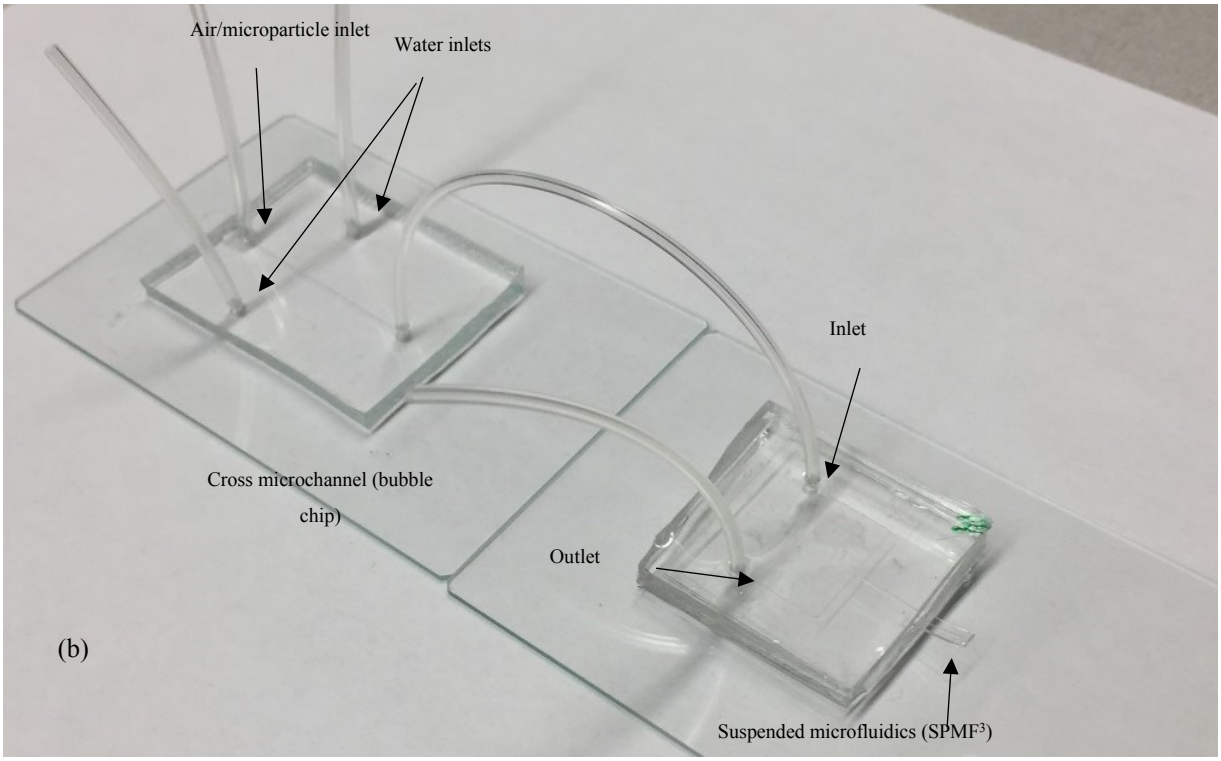


Figure 5-7. Experimental setup for microparticles and bubbles detection, a) Schematic view of complete test setup, b) Hybrid arrangement of bubble chip and SMPF^3

Five different flow rates of bubbles were generated as 5, 10, 15, 20, 25 and 30 µl/min and the experiments were repeated on three fabricated SPMF^{3}s with microcantilever dimensions of 6000×2000×600µm, length × width × thickness, respectively and an embedded microfluidic channel of 400×100µm and 200×100µm, width and depth, respectively. Two SPMF^3 were fabricated with 200×100µm microchannels but with and without a predesigned offset between the upper and lower microchannels. The third SPMF^3 was fabricated with 400×100µm microchannels and a large offset between the microchannels. The offset amount modifies the SPMF^3 deflection pattern as the micro-bubble passes through it (Figure 5-8). This can be used for biophysical studies such as deformability of bioelements and cells. Moreover, the microchannel size variation shows higher sensitivity of the SPMF^3 to flow rates in smaller microchannels.

As it is shown in Figure 5-9, each SPMF^3 design shows different reaction to the micro-bubbles flow at the same flow rate of 15 µl/min as expected and mentioned. Moreover, the microfluidics with high offset shows four peaks for each bubble which is due to turning or

deformation of bubbles at the corners between upper and lower microchannels. However, the SPMF^3 with small offset has two peaks and the SPMF^3 without offset does not have a sharp peak except when the bubble speed is more than 15 µl/min which is due to high momentum. During this experiment, the microcantilever lateral deflection was also measured which shows higher lateral deflection as the offset increases. This lateral deflection is due to the lateral deformation of the micro-bubbles at the microchannel corners which create both vertical and lateral forces.

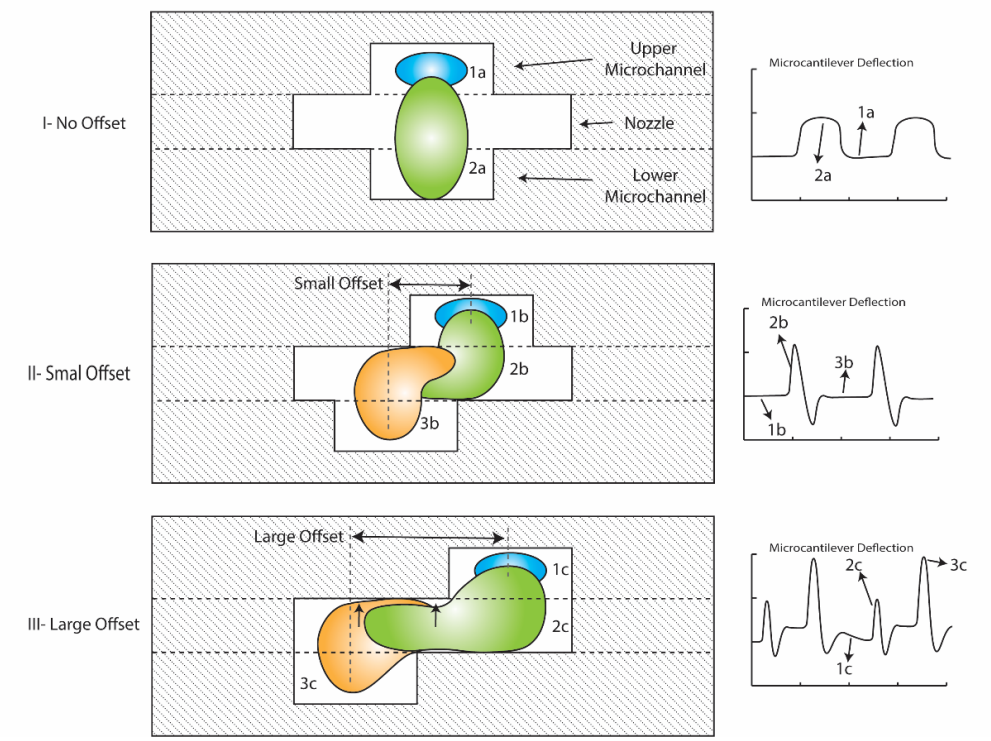


Figure 5-8. Cross sectional view of the SPMF^3 with different offsets between upper and lower microchannels; micro-bubbles at different times are shown schematically

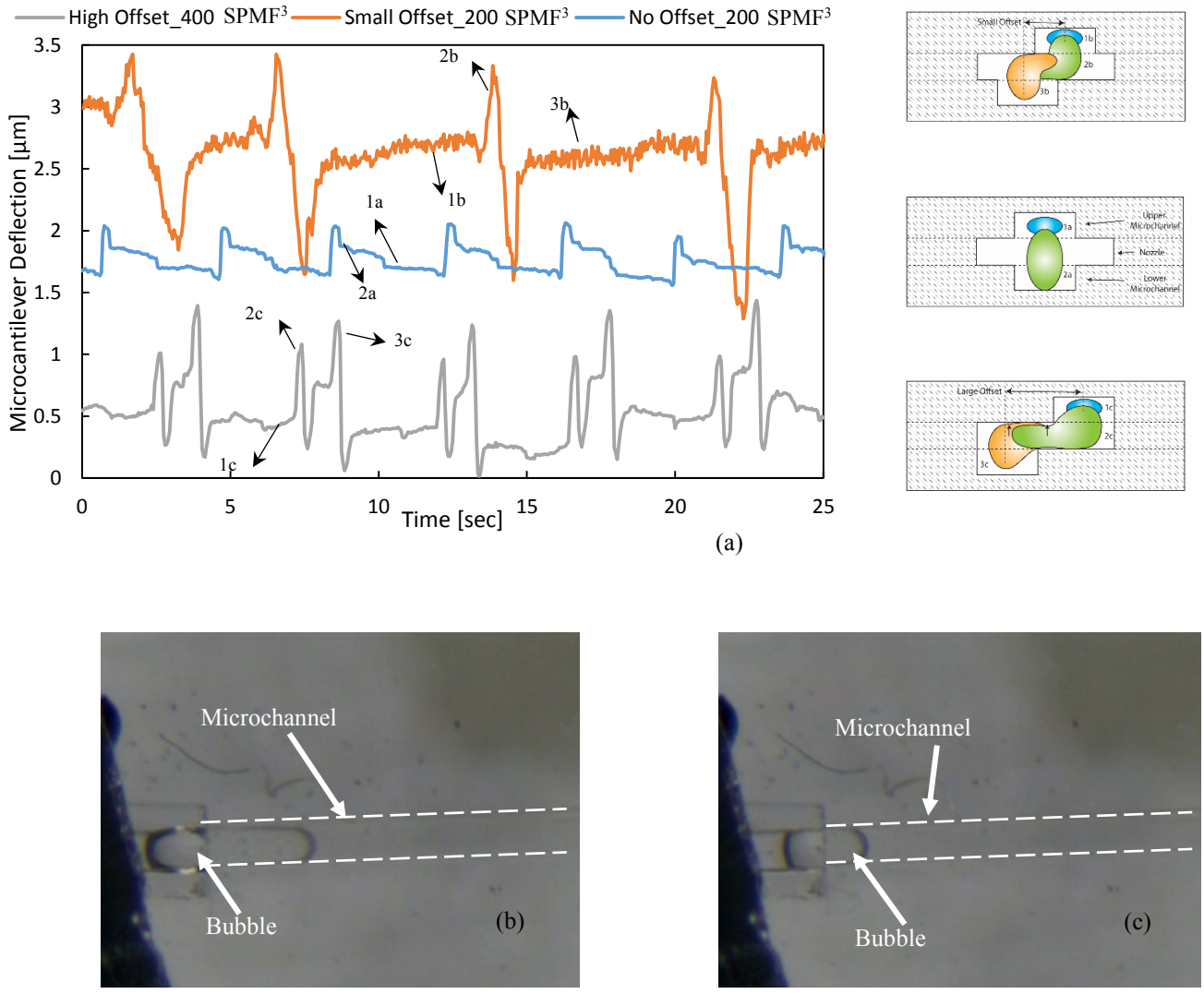


Figure 5-9. a) Micro-bubbles flow test results in three different SPMF^3 b) Micro-bubbles flow inside microfluidics, c) Micro-bubbles deformation at nozzle area (- - - channel border)

In order to further investigate the offset effect and flow rate consequence on the bubbles flow, the peak to peak deflection of microcantilever was depicted against flow rate in these three different SPMF^{3}s. As it is shown in Figure 5-10 increasing flow rate, magnifies the micro-bubbles momentum which affects the final peak to peak microcantilever deflection. In other words, the smaller bioparticles can be detected and analyzed with higher sensitivity using the SMPF^3 at higher flow rates. Finally, these results show the SPMF^3 sensitivity to bubbles flow and number of bubbles which can be counted with number of peaks.

In order to demonstrate the detection of rigid particles, an experiment was done using polystyrene spherical microparticles with diameter of 60µm. In order to track microparticles during the experiments, the solution was diluted to a very low concentration of particles during which, maximum 5 particles per minute were flown inside microchannel and the microcantilever deflection was recorded using the optical laser based deflection measurement system.

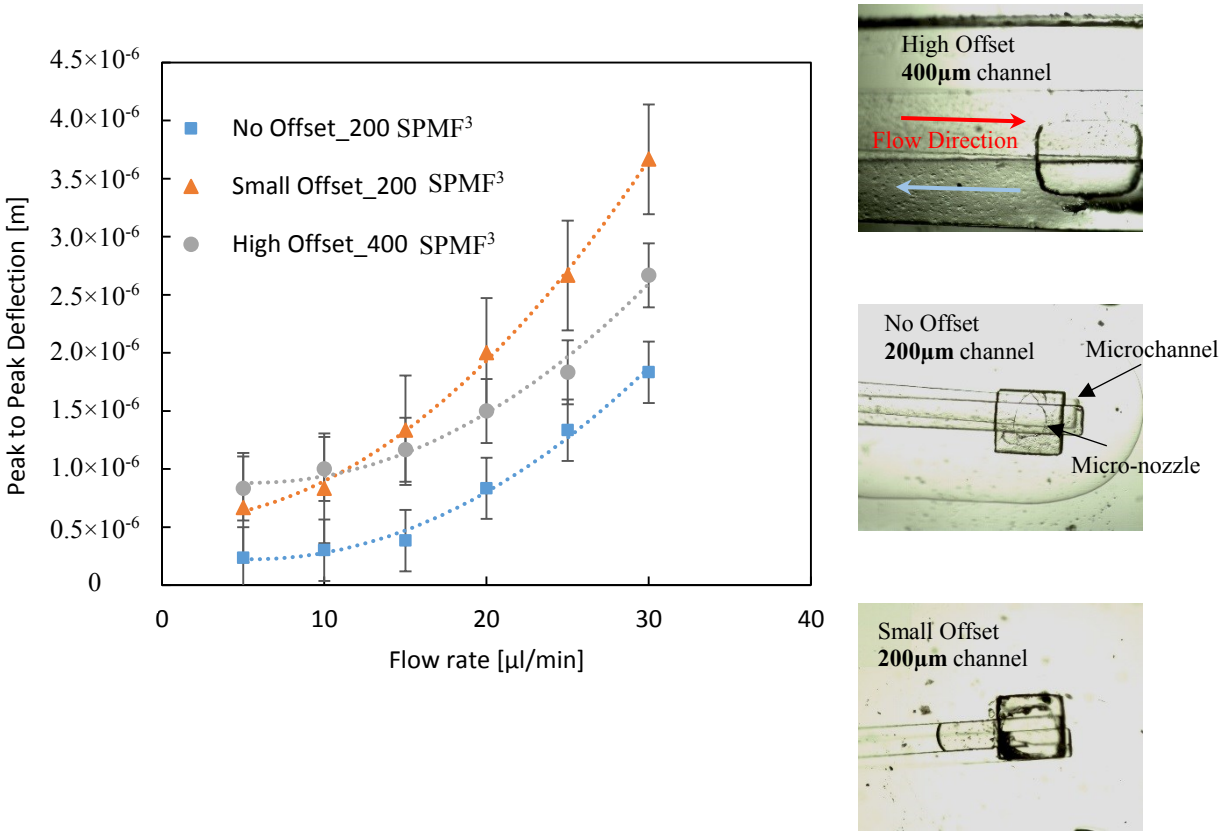


Figure 5-10. Peak to peak deflections of the three different SMPF^{3}s versus micro-bubbles flow rate

An SPMF^3 with microcantilever dimensions of 6000×2000×600µm of length, width and thickness, respectively with and an embedded microfluidic channel of 200×100µm of width and depth, respectively was employed for this experiment.

Microparticles under a flow rate of 10 and 15 µl/min were injected inside microchannel and the results are as shown in Figure 5-11. Similar to the bubbles flow, when rigid particles pass through the nozzle, a change in flow forces happens inside microchannel which results in

microcantilever deflection when particle passes from the tip of the cantilever. Studying the number, amplitude and sequence of these peaks will help in characterizing number, dimension, flow rate and speed of microparticles inside the SPMF^3. In other words, higher the peak amplitude means higher momentum force which comes from the flow direction variation and particle momentum. As it is shown here, when the flow rate is increased, the deflection peak increases which is due to higher particle momentum at the nozzle area though the blockage ratio is the same as the particle size is kept constant. This means that the SPMF^3 is very sensitive to any flow force variation at nozzle area which helps in detection of smaller microparticles. Even if they are not easily detectable at certain flow rates, increasing the particles speed in microchannel rises the peak amplitude and subsequently the sensitivity to smaller particles. On the other hand, based on the simulation results changing the blockage ratio at nozzle representing bigger/smaller microparticles will modify the deflection peak amplitude which results in particle size discrimination and sorting. Moreover, the microcantilever has a constant deflection at each flow rate which is shown here as a mean line and this indicates the particles speed during their flow in microchannel. Thus, the mean deflection and peak deflection amplitude deliver crucial information about physical properties of the elements under study.

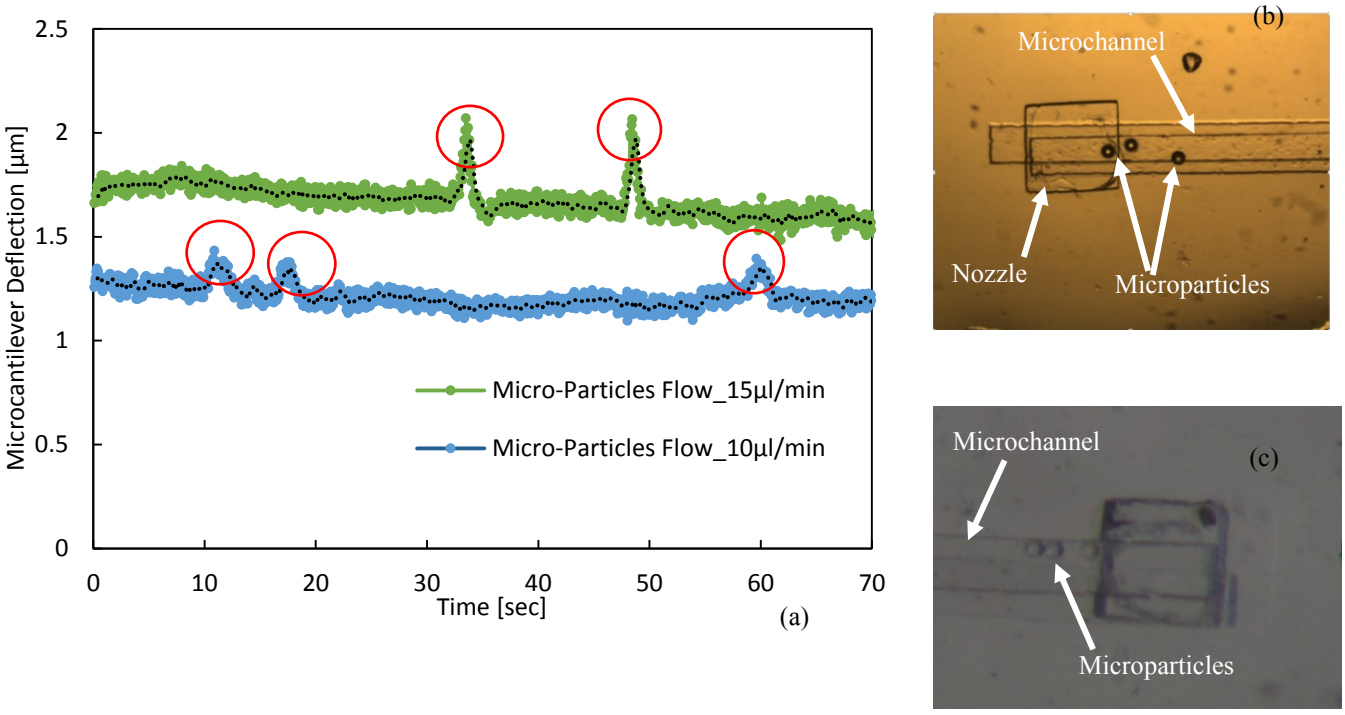


Figure 5-11. a) Results of microparticles detection and analysis experiment using the SPMF^3, b) Microparticles flow inside microchannel, c) Microparticles at microchannel wall

5.6. Conclusions

Bioparticles detection, sizing, sorting and other biophysical analyses are done by current commercial cytometers which work based on integration of optical and electrical techniques [118]. However, these machines which are bulky, consume high sample amounts and are non-portable. To overcome these issues, microsystems concept has been thoroughly considered by many researchers. In this regard, new points of view to current microstructures and introducing an innovative SPMF^3 platform with flow plane orthogonal to neutral plane is proved as a promising solution. The SPMF^3 detects and studies microparticles through monitoring deflections caused by fluid flow forces applied to the microcantilever. Monitoring number and amplitude of these deflections, one can count and differentiate various microparticles.

According to the simulation results, the SPMF^3 sensitivity can be modified using different micro-nozzle dimensions in order to detect and count various bioparticles. In order to verify design and sensitivity of the SPMF^3, 60 µm polystyrene microparticles as rigid particles and air

bubbles as flexible particles with different flow rates were tested. According to these results, flowing particles and bubbles through nozzle applies pulsating forces which consequently deflect the microcantilever. Monitoring these deflection peaks and amplitudes can identify the count, size and elastic properties of the injected microparticles and bubbles.

Chapter 6

Conclusions and Future Works

In this study, the concept of a 3D suspended microfluidics for sensitive diagnostic applications has been developed and verified. In order to reach this biodiagnostic platform, first the suspended microfluidics was designed to introduce an innovative transduction principle for bioanalysis applications which is the flow force. Then, the applied forces were intensified through design modifications in order to reach the required sensitivity for biodiagnostics. Secondly, the fabrication process was improved in order to implement all of the designed features properly. Finally, different sets of experiments such as fluid properties analysis and microparticles detection were designed to validate the capabilities of the suspended microfluidics as summarized below.

6.1. Summary and conclusions

1- Design of a suspended microfluidics for biadiagnostic applications without external excitation was one of the main achievements of the present thesis. The SPMF^3 employs flow forces as transduction principle to extract bioelements properties. In order to improve the sensitivity of SPMF^3, the microchannel plane was modified to be perpendicular to the microcantilever bending plane. According to the detail simulation results, this modification improves the sensitivity up to five times in comparison with other systems of its kind under the same condition.

Embedding a micro-nozzle between the two microchannel layers provides this capability to improve the SPMF^3 sensitivity as required according to the bioelements under study. A detailed finite element simulation shows that lowering the micro-nozzle dimensions increases the range of microcantilever deflections up to eighteen times in comparison with

an SPMF^3 platform with higher micro-nozzle dimensions which makes this platform highly sensitive. This specification as well as microchannel dimensions offers a set of SPMF^{3}s which can be customized for a variety of biodiagnostic applications.

2- Developing a fabrication process for the 3D suspended microfluidics was a challenging objective which has not been done earlier to the best of our knowledge and was accomplished through 3-layer process development. The main issue during microfabrication was lack of bonding adhesion among PDMS layers without which the SPMF^3 does not function properly. In order to avoid the leakage among fabricated layers, curing process temperature was optimized to 65C to have more stickiness at the surface of the PDMS layers. Moreover, two small channels were placed on the edge of microchannel layers to removed blocked air between layers during the bonding process. These two steps removed the leakage issue completely which is necessary to perform any experiment.

3- Optimizing different design and fabrication parameters such as microchannel offset for higher sensitivity is another aspect of the designed SPMF^3 which is implemented during fabrication processes. Since the transduction principle of this biodiagnostic platform is the flow force, any variation in flow direction creates additional forces which can be employed for sensing applications. Here, three different offsets were created during aligning PDMS layers in bonding process and their effects were studied on micro-bobble flow. According to these experimental results, the microchannel with higher offset creates sharper picks when a microbubble passes through the nozzle. Moreover, this offset creates some lateral forces and consequently lateral displacements in microcantilever which increase with the amount of offset.

4- Measuring steady and pulsatile flow rates were performed in this thesis as another application of the SPMF^3. In these experiments, steady flow rates as low as 10µl/min were detected which can be optimized for even nanoliter flow rate measurements through the design parameters mentioned here. Pulsatile flow detection and measurements performed

using a peristaltic pump demonstrates that the SPMF^3 is sensitive to pulsating load and can be employed for transient biodiagnostic applications.

5- Biofluids and chemical mixtures are specified with variations in fluid properties such as viscosity and density. However, either viscosity or density has different or sometimes opposite effects on the micro-measurement devices. In this thesis, these opposite effects were discussed and simulated in details and a comprehensive parameter was introduced. Kinematic viscosity considers both inter-molecular forces as well as molecular mass in the fluid specification which is the only parameter for fluid process detection, especially in complex fluids. According to the experimental, simulation and theoretical results, the SPMF^3 design provides a unique platform among other micro-viscometers which can measure and specify complex and regular fluids according to their kinematic viscosity. Using the kinematic viscosity in biodiagnostic applications, there is no need for measuring and specifying other properties such as dynamic viscosity and density.

6- Detecting microparticles and measuring their properties such as dimension, speed and count using the suspended microfluidics were successfully performed using the SPMF^3. According to the experimental results, polystyrene beads with 60um diameter were detected and counted. Moreover, the SPMF^3 deflection peak during microparticles flow is proportional to flow rate and particle diameter. Smaller bioelements can be detected using the same SPMF^3 with higher flow rates.

6.2. Future works

The thesis objective was to develop a sensitive platform for biodiagnostic applications. Several steps have been accomplished to design, fabricate and validate a reliable platform for bioelement analysis. However, as the research never ends, there are some recommendations for future investigations.

1- The sensitivity of the suspended microfluidics can be further studied through detailed simulations involving solid microcantilever, microfluidic interaction forces and microparticle dynamics. The sensitivity can still be improved through geometrical

modifications of microchannel and nozzle such as addition of nozzle and other restrictive elements. Having obtained this numerical model, an optimized system design according to each application can be developed.

2- In the current study, microparticles solution was diluted and its concentration was reduced to prevent particles clogging. The clogging issue that happens due to high particles flow rate and lack of appropriate surface treatment of microchannel can be considered in future studies. Then higher particles flow rate can be inserted in the $SPMF^3$ platform using suitable surface treatment processes such as Teflon coating or employment of different surfactant coatings such as TWEEN20. This step is important for studying microparticles with high mass flow rate.

3- Study of mechanical properties of bioparticles such as flexibility of cells can be done through introduction of predefined offsets among the $SPMF^3$ layers. As it was mentioned in conclusions, offset between microchannel layers creates vertical and lateral forces/displacements on the microcantilever. Measuring both vertical and lateral deflections of flexible and rigid microparticles flow should provide some differences in the final deflection pattern by which mechanical properties of bioelements are revealed.

4- Detection and measurement of bioelements mass flow rate can be done using an $SPMF^3$ platform in which the microchannel walls are functionalized with appropriate receptors. This technique, namely labeling, have been employed in other biosensors such microcantilevers, SMR and SPMF. Having modified the 3D suspended microfluidic design for the stress deflection measurement, one can detect the bioelements mass flow rate through cantilever deflections of the $SPMF^3$. Thus, sensitivity of the $SPMF^3$ platform can be compared with similar biosensors such as SMR and SPMF.

5- In order to define practical applications for the newly developed $SPMF^3$, performing bioelements detection and analysis experiments in cooperation with a biologist is recommended in future steps. In this regard, addition of Aspirin to blood which removes

some proteins from red blood cells and consequently modifies blood viscosity can be specified using SPMF^3 and differences in solution parameters can be extracted. As such, drug development applications can be considered as one the potential industries which may find the SPMF^3 practical.

6- Integration of the laser deflection measurement system or other measurement techniques into a single lab-chip along with the SPMF^3 will reduce the experiment time and paves the way for possible commercial applications as a single chip.

References

[1] J. Arlett, E. Myers and M. Roukes, "Comparative advantages of mechanical biosensors," *Nature Nanotechnology,* vol. 6, pp. 203-215, 2011.

[2] E. Bianconi, A. Piovesan, F. Facchin, A. Beraudi, R. Casadei, F. Frabetti, L. Vitale, M. C. Pelleri, S. Tassani, F. Piva, S. Perez-Amodio, P. Strippoli and S. Canaider, "An estimation of the number of cells in the human body," *Annals of Human Biology,* vol. 40, pp. 463-471, 2013.

[3] H. Zhang, C. H. Chon, X. Pan and D. Li, "Methods for counting particles in microfluidic applications," *Microfluid Nanofluid,* vol. 7, p. 739–749, 2009.

[4] C. Lancaster, M. Kokoris, M. Nabavi, J. Clemmens, P. Maloney, J. Capadanno, J. Gerdes and C. Battrell, "Rare cancer cell analyzer for whole blood applications: Microcytometer cell counting and sorting subcircuits," *Methods,* vol. 37, p. 120–127, 2005.

[5] J. Tamayo, P. M. Kosaka, J. J. Ruz, A. S. Paulo and M. Calleja, "Biosensors based on nanomechanical systems," *Chem. Soc. Rev,* vol. 42, pp. 1287--1311, 2013.

[6] R. W. DeBlois and C. P. Bean, "Counting and sizing of submicron particles by the resistive pulse technique," *Rev Sci Instrum.,* vol. 41, p. 909–915, 1970.

[7] X. Wu, C. K. Chon, Y. W. Y. and D. Li, "Simultaneous particle counting and detecting on a chip," *Lab Chip,* vol. 8, p. 1943–1949, 2008.

[8] J. Zhe, A. Jagtiani, P. Dutta, J. Hu and J. Carletta, "A micromachined high throughput Coulter counter for bioparticle detection and counting," *J Micromech Microeng,* vol. 17, p. 304–313, 2007.

[9] S. Gawad, L. Schild and P. Renaud, "Micromachined impedance spectroscopy flow cytometer for cell analysis and particle sizing," *Lab Chip,* vol. 1, p. 76–82, 2001.

[10] D. Wood, S. Oh, S. Lee, H. Soh and A. Cleland, "High-bandwidth radio frequency Coulter counter.," *Appl Phys Lett,* vol. 87, p. 184106, 2005.

[11] S. Gawad, K. Cheung, U. Seger, A. Bertsch and P. Renaud, "Dielectric spectroscopy in a micromachined flow cytometer: theoretical and practical considerations," *Lab Chip,* vol. 4, p. 241–251, 2004.

[12] K. Cheung, S. Gawad and P. Renaud, "Impedance spectroscopy flow cytometry: on-chip label-free cell differentiation," *Cytometry A,* vol. 65A, p. 124–132, 2005.

[13] T. Sun and H. Morgan, "Single-cell microfluidic impedance cytometry: a review," *Microfluid Nanofluid,* vol. 8, p. 423–443, 2010.

[14] S. Gawad, T. Sun, N. Green and H. Morgan, "Impedance spectros- copy using maximum length sequences: application to single cell analysis," *Rev Sci Instrum,* vol. 78, p. 054301, 2007.

[15] T. Sun, C. Berkel, N. Green and H. Morgan, "Digital signal processing methods for impedance microfluidic cytometry," *Microfluid Nanofluid,* vol. 6, p. 179–187, 2009.

[16] R. Rodriguez-Trujillo, C. Mills, J. Samitier and G. Gomila, "Lowcost micro-Coulter counter with hydrodynamic focusing," *Microfluid Nanofluid,* vol. 3, p. 171–176, 2007.

[17] R. Rodriguez-Trujillo, O. Castillo-Fernandez, M. Garrido, M. Arundell, A. Valencia and G. Gomila, "High-speed particle detection in a micro-Coulter counter with two-dimensional adjustable aperture," *Biosens Bioelectron,* vol. 24, p. 290–296, 2008.

[18] C. Bernabini, D. Holmes and H. Morgan, "Micro-impedance cytometry for detection and analysis of micron-sized particles and bacteria," *Lab on a Chip,* vol. 11, p. 407–412, 2011.

[19] L. Sohn, O. Saleh, G. Facer, A. Beavis, R. Allan and D. Notterman, "Capacitance cytometry: measuring biological cells one by one.," *Proc Natl Acad Sci,* vol. 97, p. 10687–10690, 2000.

[20] N. Pamme, R. Koyama and A. Manz, "Counting and sizing of particles and particle agglomerates in a microfluidic device using laser light scattering: application to a particle-enhanced immunoassay," *Lab Chip,* vol. 3, p. 187–192, 2003.

[21] Q. Xiang, X. Xuan, B. Xu and D. Li, "Multi-functional particle detection with embedded optical fibers in a poly(dimethylsiloxane)," *Chip. Instrum Sci Technol,* vol. 33, p. 597–607, 2005.

[22] A. Kummrow, J. Theisen, M. Frankowski, A. Tuchscheerer, H. Yildirim, K. Brattke, M. Schmidt and J. Neukammer, "Microfluidic structures for flow cytometric analysis of hydrodynamically focused blood cells fabricated by ultraprecision micromachining," *Lab Chip,* vol. 9, p. 972–981, 2009.

[23] M. McClain, C. Culbertson, S. C. Jacobson and J. Ramsey, "Flowcytometry of Escherichia coli on microfluidic devices," *Anal. Chem.,* vol. 73, p. 5334–8, 2001.

[24] Y.-C. Tung, M. Zhang, C.-T. Lin, K. Kurabayashi and S. Skerlos, "PDMS-based opto-fluidic micro flow cytometer with two-color, multi-angle fluorescence detection capability using PIN photodiodes," *Sensors Actuators B,* vol. 98, p. 356–367, 2004.

[25] X. Mao, S.-C. S. Lin, C. Dong and T. J. Huang, "Single-layer planar on-chip flow cytometer using microfluidic drifting based three-dimensional (3D) hydrodynamic focusing," *Lab on a Chip,* vol. 9, p. 1583–1589, 2009.

[26] C. Simonnet and A. Groisman, "High-Throughput and High-Resolution Flow Cytometry in Molded Microfluidic Devices," *Anal. Chem.,* vol. 78, pp. 5653-5663, 2006.

[27] D. E. Jaalouk and J. Lammerding, "Mechanotransduction gone awry," *Nature reviews Molecular cell biology,* vol. 10, no. 1, pp. 63-73, 2009.

[28] A. W. Orr, B. P. Helmke, B. R. Blackman and M. A. Schwartz, "Mechanisms of mechanotransduction," *Developmental cell,* vol. 10, no. 1, pp. 11-20, 2006.

[29] N. Wang, J. D. Tytell and D. E. Ingber, "Mechanotransduction at a distance: mechanically coupling the extracellular matrix with the nucleus," *Nature reviews Molecular cell biology,* vol. 10, no. 1, pp. 75-82, 2009.

[30] C. G. Galbraith and M. P. Sheetz, "A micromachined device provides a new bend on fibroblast traction forces," *Proceedings of the National Academy of Sciences,* vol. 94, no. 17, pp. 9114-9118, 1997.

[31] M. T. A. Saif, C. R. Sager and S. Coyer, "Functionalized biomicroelectromechanical systems sensors for force response study at local adhesion sites of single living cells on substrates," *Annals of Biomedical Engineering,* vol. 31, no. 8, pp. 950-961, 2003.

[32] S. Yang and T. Saif, "Reversible and repeatable linear local cell force response under large stretches," *Experimental cell research,* vol. 305, no. 1, pp. 42-50, 2005.

[33] J. Park, J. Ryu, S. K. Choi, E. Seo, J. M. Cha, S. Ryu and S. H. Lee, "Real-time measurement of the contractile forces of self-organized cardiomyocytes on hybrid biopolymer microcantilevers," *Analytical chemistry,* vol. 77, no. 20, pp. 6571-6580, 2005.

[34] U. Zimmermann and E. Steudle, "Physical aspects of water relations of plant cells," *Advances in Botanical Research,* vol. 6, pp. 45-117, 1979.

[35] N. P. Money and F. M. Harold, "Extension growth of the water mold Achlya: interplay of turgor and wall strength," *Proceedings of the National Academy of Sciences,* vol. 89, no. 10, pp. 4245-4249, 1992.

[36] N. P. Money and F. M. Harold, "Two water molds can grow without measurable turgor pressure," *Planta,* vol. 190, no. 3, pp. 426-430, 1993.

[37] R. Benkert, G. Obermeyer and F.-W. Bentrup, "The turgor pressure of growing lily pollen tubes," *Protoplasma,* vol. 198, no. 1, pp. 1-8, 1997.

[38] J. Blewett, K. Burrows and C. Thomas, "A micromanipulation method to measure the mechanical properties of single tomato suspension cells," *Biotechnology letters,* vol. 22, no. 23, pp. 1877-1883, 2000.

[39] L. Wang, D. Hukin, J. Pritchard and C. Thomas, "Comparison of plant cell turgor pressure measurement by pressure probe and micromanipulation," *Biotechnology letters,* vol. 28, no. 15, pp. 1147-1150, 2006.

[40] N. P. Money, "Biomechanics of invasive hyphal growth," in *Biology of the Fungal Cell*, vol. 8, NewYork, Springer, 2001, pp. 3-17.

[41] M. G. Mardasi and M. Packirisamy, "Lab-On-Chip for Ex-Vivo study of morphogenesis of tip growing cells of pollen tube," PhD Thesis, Cocordia University, 2013.

[42] T. Braun, "Micromechanical mass sensors for biomolecular detection in a physiological environment," *Phys. Rev. E: Stat. Phys., Plasmas, Fluids, Relat. Interdiscip. Top.,* vol. 72, no. 031907, 2005.

[43] J. Tamayo, A. Humphris, A. Malloy and M. Miles, "Chemical sensors and biosensors in liquid environment based on microcantilevers with amplified quality factor," *Ultramicroscopy,* vol. 86, pp. 167-173, 2001.

[44] D. Ramos, "Arrays of dual nanomechanical resonators for selective biological detection," *Anal. Chem.,* vol. 81, p. 2274–2279, 2008.

[45] P. S. Waggoner, M. Varshney and H. G. Craighead, "Detection of prostate specific antigen with nanomechanical resonators," *Lab Chip,* vol. 9, p. 3095–3099, 2009.

[46] A. Naik, M. Hanay, W. Hiebert, X. Feng and M. Roukes, "Towards single-molecule nanomechanical mass spectrometry," *Nat. Nanotechnol.,* vol. 4, p. 445–450, 2009.

[47] J. H. Lee, K. S. Hwang, J. Park, K. H. Yoon, D. S. Yoon and T. S. Kim, "Immunoassay of prostate-specific antigen (PSA) using resonant frequency shift of piezoelectric nanomechanical microcantilever," *Biosensors and Bioelectronics,* vol. 20, no. 10, p. 2157–2162, 2005.

[48] B. Ilic, D. Czaplewski, M. Zalalutdinov, H. G. Craighead, P. Neuzil, C. Campagnolo and C. Batt, "Single cell detection with micromechanical oscillators," *Journal of Vacuum Science & Technology B,* vol. 19, p. 2825–2828.

[49] T. P. Burg, M. Godin, S. M. Knudsen, W. Shen, G. Carlson, J. S. Foster, K. Babcock and S. R. Manalis, "Weighing of biomolecules, single cells and single nanoparticles in fluid," *Nature,* vol. 446, pp. 1066-1069, 2007.

[50] A. K. Bryan, V. C. Hecht, S. Wenjiang, P. Kristofor, W. H. Grover and S. R. Manalis, "Measuring single cell mass, volume, and density Measuring single cell mass, volume, and density," *Lab on a Chip,* vol. 14, pp. 569-576, 2014.

[51] J. Lee, R. Chunara, W. Shen, K. Payer, K. Babcock, T. P. Burg and S. R. Manalis, "Suspended microchannel resonators with piezoresistive sensors," *Lab Chip,* vol. 11, p. 645–651, 2011.

[52] H. Sadabadi and M. Packirisamy, "Nano-integrated Polymeric Suspended Microfluidic Platform for Ultra-Sensitive Bio-Molecular Recognition," PhD Thesis, Concordia University, 2013.

[53] P. S. Waggoner and H. G. Craighead, "Micro- and nanomechanical sensors for environmental, chemical, and biological detection," *Lab on a Chip,* vol. 7, p. 1238–1255, 2007.

[54] J. Arlett, E. Myers and M. Roukes, "Comparative advantages of mechanical biosensors," *Nat. Nanotechnol.,* vol. 6, p. 203–215, 2011.

[55] M.-I. Mohammed and M. P. Y. Desmulliez, "Lab-on-a-chip based immunosensor principles and technologies for the detection of cardiac biomarkers: a review," *Lab on a Chip,* vol. 11, p. 569–59, 2011.

[56] R. Datar, "Cantilever sensors: nanomechanical tools for diagnostics," *MRS Bull.,* vol. 34, p. 449–454, 2009.

[57] J. Fritz, "Cantilever biosensors," *Analyst,* vol. 133, p. 855–863, 2008.

[58] R. Raiteri, M. Grattarola, H. J. Butt and P. Skla´dal, "Micromechanical cantilever-based biosensors," *Sens. Actuators,* vol. 79, p. 115–126, 2001.

[59] J. Fritz, "Translating biomolecular recognition into nanomechanics," *Science,* vol. 288, p. 316–318, 2000.

[60] K. Gruber, "Cantilever Array Sensors Detect Specific Carbohydrate–Protein Interactions with Picomolar Sensitivity," *ACS Nano,* vol. 5, p. 3670–3678, 2011.

[61] J. W. Ndieyra, "Nanomechanical detection of antibiotic mucopeptide binding in a model for superbug drug resistance," *Nat. Nanotechnol,* vol. 3, pp. 691-696, 2008.

[62] G. Wu, R. H. Datar, K. M. Hansen, T. Thundat, R. J. Cote and A. Majumdar, "Bioassay of prostate-specific antigen (PSA) using microcantilevers," *Nature Biotechnology ,* vol. 19, p. 856–860, 2001.

[63] C. A. Savran, S. M. Knudsen, A. D. Ellington and S. R. Manalis, "Micromechanical Detection of Proteins Using Aptamer-Based Receptor Molecules," *Analytical Chemistry,* vol. 76, no. 11, p. 3194–3198, 2004.

[64] N. Backmann, C. Zahnd, F. Huber, A. Bietsch, A. Plückthun, H.-P. Lang, H.-J. Güntherodt, M. Hegner and C. Gerber, "A label-free immunosensor array using single-chain antibody fragments," *Proceedings of the National Academy of Sciences of the United States of America,* vol. 102, no. 41, p. 14587–14592, 2005.

[65] B. Ilic, D. Czaplewski, M. Zalalutdinov, H. G. Craighead, P. Neuzil, C. Campagnolo and C. Batt, "Single cell detection with micromechanical oscillators," *Journal of Vacuum Science & Technology B,* vol. 19, p. 2825–2828.

[66] G. E. Trope, G. D. Lowe, B. M. McArdle, J. T. Douglas, C. D. Forbes, C. M. Prentice and W. S. Foulds, "Abnormal blood viscosity and haemostasis in long-standing retinal vein occlusion," *British Journal of Ophthalmology,* vol. 63, p. 137–142, 1983.

[67] Y. Cho and K. Kensey, "Effects of the non-Newtonian viscosity of blood on flows in a diseased arterial vessel. Part 1: Steady flows," *Biorheology,* vol. 28, pp. 241-262, 1991.

[68] Y. Cho, J. Hartnett and W. Lee, "Non-Newtonian viscosity measurements in the intermediate shear rate range with the falling-ball viscometer," *Journal of non-Newtonian fluid mechanics,* vol. 15, pp. 61-74, 1984.

[69] B. A. Averill and P. Eldredge, in *Principles of General Chemistry*, Creative Commons, 2012, p. 407.

[70] J.E. Sader, "frequency response of cantilever beams immersed in viscous fluids with applications to the atomic force microscope," *Journal of Applied Physics,* vol. 84, pp. 64-76, 1998.

[71] H. Muramatsu, E. Tamiya and I. Karube, "Computation of equivalent circuit parameters of quartz crystals in contact with liquids and study of liquid properties," *Analytical Chemistry,* vol. 60, pp. 2142-2146, 1988.

[72] D. Angelescu, H. Chen, J. Jundt, H. Berthet, B. Mercier and F. Marty, "Highly integrated microfluidic sensors," in *Proceedings of SPIE*, San Diego, USA, 2008.

[73] B. Jakoby, M. Scherer, M. Buskies and H. Eisenschmid, "An automotive engine oil viscosity sensor," *IEEE Sensors Journal,* vol. 3, pp. 562-568, 2003.

[74] L. Matsiev, "Application of flexural mechanical resonators to high throughput liquid characterization," in *2000 IEEE Ultrasonics Symposium*, San Juan, Puerto Rico, 2000.

[75] S. Wang, "Engine oil condition sensor: method for establishing correlation with total acid number," *Sensors and Actuators B: Chemical,* vol. 86, pp. 122-126, 2002.

[76] I. Goubaidoulline, J. Reuber, F. Merz and D. Johannsmann, "Simultaneous determination of density and viscosity of liquids based on quartz crystal resonators covered with nanoporous alumina," *Journal of Applied Physics,* vol. 98, pp. 014305-1–014305-4, 2005.

[77] F. Herrmann, D. Hahn and S. Buttgenback, "Separate determination of liquid density and viscosity with sagittally corrugated Love-mode sensors," *Sensors and Actuators A Physical,* vol. 78, pp. 99-107, 1999.

[78] K. Durdag, "Solid state acoustic wave sensors for real-time in-line measurement of oil viscosity," *Sensor Review,* vol. 28, pp. 68-73, 2008.

[79] A. Agoston, C. Otsch and B. Akoby, "Viscosity sensors for engine oil condition monitoring—Application and interpretation of results," *Sensors and Actuators A Physical,* vol. 121, pp. 327-332, 2005.

[80] L. Matsiev, "Application of flexural mechanical resonators to high throughput liquid characterization," in *Proceedings of 2000 IEEE Ultrasonics Symposium*, San Juan, Puerto Rico, 2000.

[81] B. Martin, "Viscosity and density sensing with ultrasonic plate waves," *Sensors and Actuators A: Physical,* vol. 22, pp. 704-708, 1990.

[82] S. Wenzel, B. Martin and R. White, "Generalized Lamb-wave multisensor," in *Proceedings of IEEE Ultrasonics Symposium* , Chicago, USA, 1988.

[83] S. Wolf and R. Tauber, Silicon Processing for the VLSI Era, Sunset Beach, California: Lattice Press, 1986.

[84] I. Puchades and L. F. Fuller, "A Thermally Actuated Microelectromechanical (MEMS) Device for Measuring Viscosity," *Journal of Microelectromechanical Systems,,* vol. 20, pp. 601-609, 2011.

[85] W. Shih, X. Li, H. Gu, W. Shih and I. a. Aksay, "Simultaneous liquid viscosity and density determination with piezoelectric unimorph cantilevers," *Journal of Applied Physics,* vol. 89, p. 1497, 2001.

[86] T. Naik, E. Longmire and S. Mantell, "Dynamic response of a cantilever in liquid near a solid wall," *Sensors and Actuators,* vol. 102, pp. 240-245, 2003.

[87] G. Rezazadeh, M. Ghanbari and I. Mirzaee, "Simultaneous Measurement of Fluids viscosity and density using a microbeam," in *International Conference on Perspective Technologies and Methods in MEMS Design*, Lviv-Polyana, Ukraine , 2009.

[88] X. Huang, S. Li, J. Schultz, Q. Wang and Q. Lin, "Mems sensor for continuous monitoring of glucose in Subcutaneous Tissue," in *IEEE 22nd International Conference on Micro Electro Mechanical Systems*, Sorrento, Italy, 2009.

[89] N. Belmiloud, I. Dufour, L. Nicu, A. Colin and J. Pistre, "Vibrating Microcantilever used as a viscometer and microrheometer," in *Proceedings of IEEE sensors*, Daegu, Korea, 2006.

[90] C. Harrison, A. Fornari, C. Hua, S. Ryu, A. R. Goodwin, K. Hsu, F. Marty and B. Mercier, "A microfluidic MEMS sensor for the measurement of density and viscosity at high pressure," *SPIE Proceedings,* vol. 6465, p. 64650U, 2007.

[91] E. Reichel, C. Riesch, F. Keplinger, B. Jakoby, I. Microelectronics and J. Kepler, "Remote electromagnetic excitation of miniaturized in-plane plate resonators for

sensing applications," in *IEEE International Frequency Control Symposium*, Honolulu, Hawaii, 2008.

[92] K. A. Ronaldson, A. D. Fitt, A. R. H. Goodwin and W. A. Wakeham, "Transversely Oscillating MEMS Viscometer: The "Spider"," *International Journal of Thermophysics,* vol. 27, no. 6, p. 1677–1695, 2006.

[93] A. Agoston, F. Keplinger and B. Jakoby, "Evaluation of a Vibrating Micromachined Cantilever Sensor for Measuring the Viscosity of Complex Organic Liquids," *Sensors and Actuators A,* vol. 123, pp. 82-86, 2005.

[94] P. Galambos and F. Forster, "An optical micro-fluidic viscometer," in *Micro-Electro-Mechanical Systems (MEMS), ASME International Mechanical Engineering Congress and Exposition*, New York, USA, 1998.

[95] M. Andrews and P. Harris, "Damping and gas viscosity measurements using a microstructure," *Sensors and Actuators: A. Physical,* vol. 49, pp. 103-108, 1995.

[96] M. M. Lara and C. Atkinson, "Theoretical model on the interaction of a vibrating beam and the surrounding viscous fluid with applications to density and viscosity sensors," in *Proceedings of IEEE Sensors*, Vienna, Austria, 2004.

[97] H. Zeng and Y. Zhao, "On-Chip Blood Viscometer Towards Point-of-Care Hematological Diagnosis," in *IEEE 22nd International Conference on Micro Electro Mechanical Systems*, Sorrento, Iraly, 2009.

[98] R. Pamies, J. G. H. Cifre, M. C. L. Martínez and J. G. Torre, "Determination of intrinsic viscosities of macromolecules and nanoparticles. Comparison of single-point and dilution procedures," *Colloid Polym Sci,* vol. 286, p. 1223–1231, 2008.

[99] C. Tanford, "Intrinsic Viscosity and Kinematic Viscosity," *J. Phys. Chem.,* vol. 59, no. 8, p. 798–799, 1955.

[100] A. Dinsdale and F. Moore, Viscosity and its measurement, London: Chapman and Hall, 1962.

[101] D. S. Viswanath, T. K. Ghosh, D. H. L. Prasad, N. K. Dutt and K. Y. Rani, Viscosity of Liquids, Theory, Estimation, Experiment, and Data, Springer, 2007.

[102] T. v. Vliet and P. Walstra, "Relationship between viscosity and fat content of milk and cream," *Texture studies,* vol. 11, no. 1, p. 65–68, 1980.

[103] L. Beaulieua, M. Godin, O. Laroche, V. Tabard-Cossa and P. Grutter, "A complete analysis of the laser beam deflection systems used in cantilever-based systems," *Ultramicroscopy,* vol. 107, p. 422–430, 2007.

[104] K. M. Hansen and T. Thundat, "Microcantilever biosensors," *Biosensors,* vol. 37, no. 1, p. 57–64, 2005.

[105] H. Sadabadi and M. Packirisamy, "Nano-integrated Polymeric Suspended Microfluidic Platform for Ultra-Sensitive Bio-Molecular Recognition," PhD Thesis, Concordia University, 2013.

[106] C. Ziegler, "Cantilever-based biosensors," *Anal Bioanal Chem,* vol. 379, p. 946–959, 2004.

[107] J. Fritz, "Cantilever biosensors," *The Analyst,* vol. 133, p. 855–863, 2008.

[108] J. Fritz, M. Baller, H. Lang, H. Rothuizen, P. Vettiger, E. Meyer, H. J. Güntherodt, C. Gerber and J. Gimzewski, "Translating biomolecular recognition into nanomechanics," *Science,* vol. 288, pp. 316-318, 2000.

[109] P. Li and X. Li, "A single-sided micromachined piezoresistive SiO2 cantilever sensor for ultra-sensitive detection of gaseous chemicals," *J. Micromech. Microeng.,* vol. 16, p. 2539–2546, 2006 .

[110] T. Thundat, P. Oden and R. Warmack, "Microcantilever sensors," *Microscale Thermophysical Engineering,* vol. 1, pp. 185-199, 1997.

[111] S. Lee, D. Kang, Y. Je and W. Moon, "Resonant frequency variations in a piezoelectric microcantilever sensor under varying operational conditions," *J. Micromech. Microeng.,* vol. 22, p. 105035, 2012.

[112] N. V. Lavrik, C. A. Tipple, M. J. Sepaniak and P. G. Datskos, "Enhanced chemi-mechanical transduction at nanostructured interfaces," *Chemical Physics Letters,* vol. 336, pp. 371-376, 2001.

[113] M. Godin, O. Laroche, V. Tabard-Cossa, L. Beaulieu, P. Grütter and P. Williams, "Combined in situ micromechanical cantilever-based sensing and ellipsometry," *Review of Scientific Instruments,* vol. 74, no. 11, pp. 4902-4907, 2003.

[114] G. Shekhawat, S. H. Tark and V. P. Dravid, "MOSFET-Embedded microcantilevers for measuring deflection in biomolecular sensors," *Science,* vol. 311, pp. 1592-1595, 2006.

[115] E. W. S. S. a. R. W. T. Thundat, "Detection of Mercury-Vapor Using Resonating Microcantilevers," *Applied Physics Letter,* vol. 66, pp. 1695-1697, 1995.

[116] T. P. Burg, A. R. Mirza, N. Milovic, C. H. Tsau, G. A. Popescu, J. S. Foster and S. R. Manalis, "Vacuum-Packaged Suspended Microchannel Resonant Mass Sensor for Biomolecular Detection," *Journal of Microelectromechanical Systems,* vol. 15, no. 6, pp. 1466 - 1476, 2006.

[117] T. P. Burg and S. R. Manalis, "Suspended microchannel resonators for biomolecular detection," *Applied Physics Letter,* vol. 83, pp. 2698-2700, 2003.

[118] D. Huh, W. Gu, Y. Kamotani and J. B. Grotberg, "Microfluidics for flow cytometric analysis of cells and particles," *Physiol. Meas.,* vol. 26, p. R73–R98, 2005.

[119] R. Mishra, W. Grange and M. Hegner, "Rapid and Reliable Calibration of Laser Beam Deflection System for Microcantilever-Based Sensor Setups," *Journal of Sensors,* vol. Article ID 617386, 2012.

Appendix A

A.1. Experimental setup preparation and results

One of the main advantages of the fabricated microsystem is real-time detection of microparticles flow inside the microchannel. This microsystem can also be used with or without labeling agents for biodiagnostic applications. To measure the microcantilever deflections, two different techniques were considered namely, image processing and optical laser deflection measurement method. Real-time deflection measurement of suspended microchannel is obtained using an image processing method. A video will be taken by a camera which is attached to a Nikon microscope in the experimental setup (Figure A-1). Here, a syringe pump has been employed to provide different flow rates inside suspended microfluidic system. After or during taking the videos in different working conditions, the image processing code, prepared in MATLAB, will be used to measure the microcantilever deflections. During this experiment and in a real-time way, a variety of biophysical properties of the microparticles such as number and size as well as flow properties such flow rate, fluid density and dynamic viscosity can be obtained.

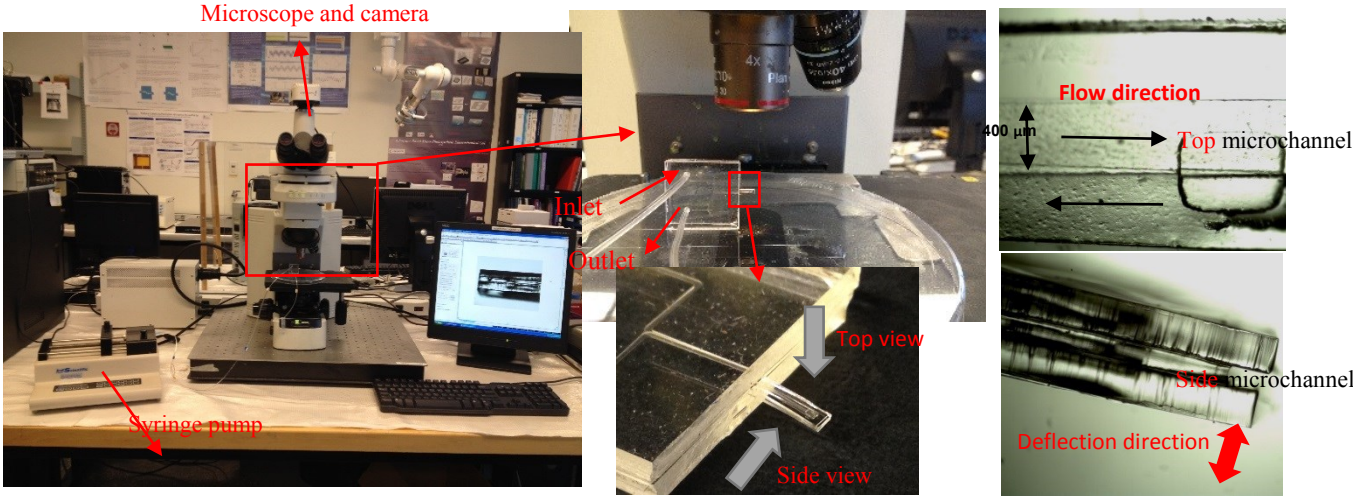


Figure A-1. Experimental setup designed for accommodating different test conditions

A.1.1. Experimental results using image processing setup

In the first experiment, the behavior analysis of the 3D suspended microfluidics (SPMF^3) against flow rate variations is performed using the image processing deflection measurement setup. Two SPMF^{3}s with microchannel dimensions of 400×100µm of width and depth respectively, and microcantilever dimensions of 6000×2000×600µm of length, width and thickness respectively, have been used. Here, the suspended microfluidics is tested under different load conditions which are provided by various syringe pump flow rates and the system response to these working conditions is shown in Figure A-2. As it is shown here, by increasing the flow rate up to 0.6 mL/min, the cantilever deflection increases with steady rate. However, for flow rates more than 0.6 mL/min, the deflection pattern has changed which shows another fluidic reaction inside microchannel. In order to stay in the safe range for the bonding strength of the SPMF^3, the fluid flow rate has been increased up to 1.6 mL/min in this experiment.

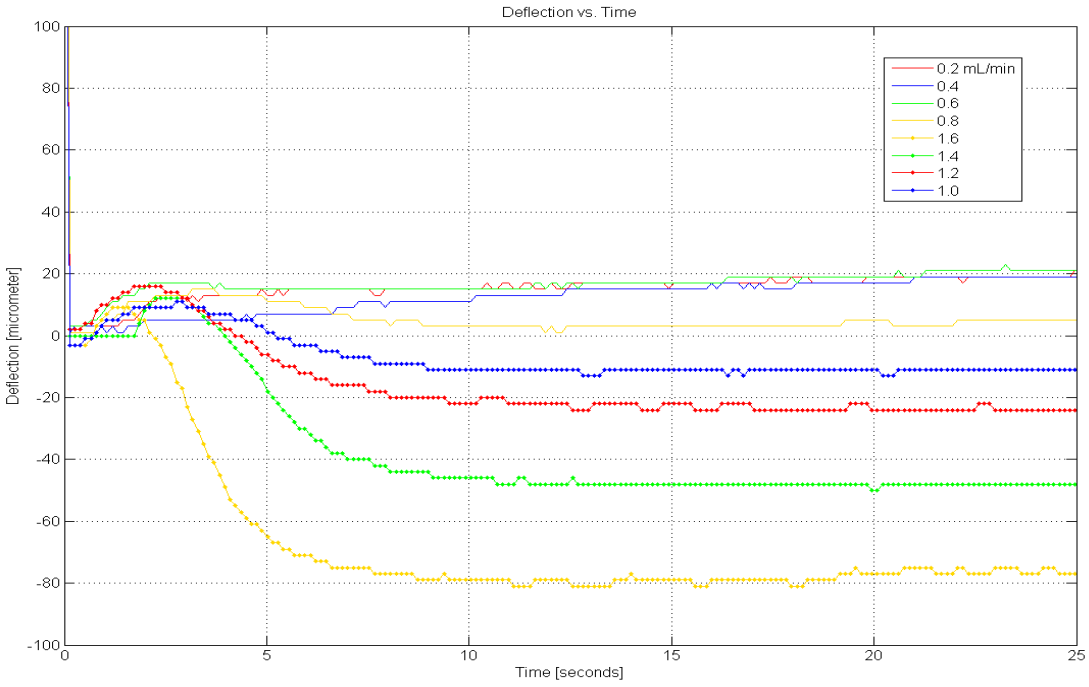


Figure A-2. Test results of the first microsystem

In order to study experimental results variation, another suspended microsystem was fabricated and tested under the same conditions. As it is shown in the following in Figure A-3, the results are different from the other microsystem tested before. This variation is due to

microchannel alignment during fabrication process. In other words, depending on design and bonding alignment of the suspended microfluidics shown in Figure *A-4*, the final deflection results and sensitivity will vary that can be used for different applications according to the parameter under study. This misalignment was intentionally made during each fabrication process in order to study the microchannel restrictions on flow forces. However, this difference does not have any effect on the application of final SPMF^3 in detection and measurement of bioelements properties. The alignment behavior analysis was discussed in chapter five in details.

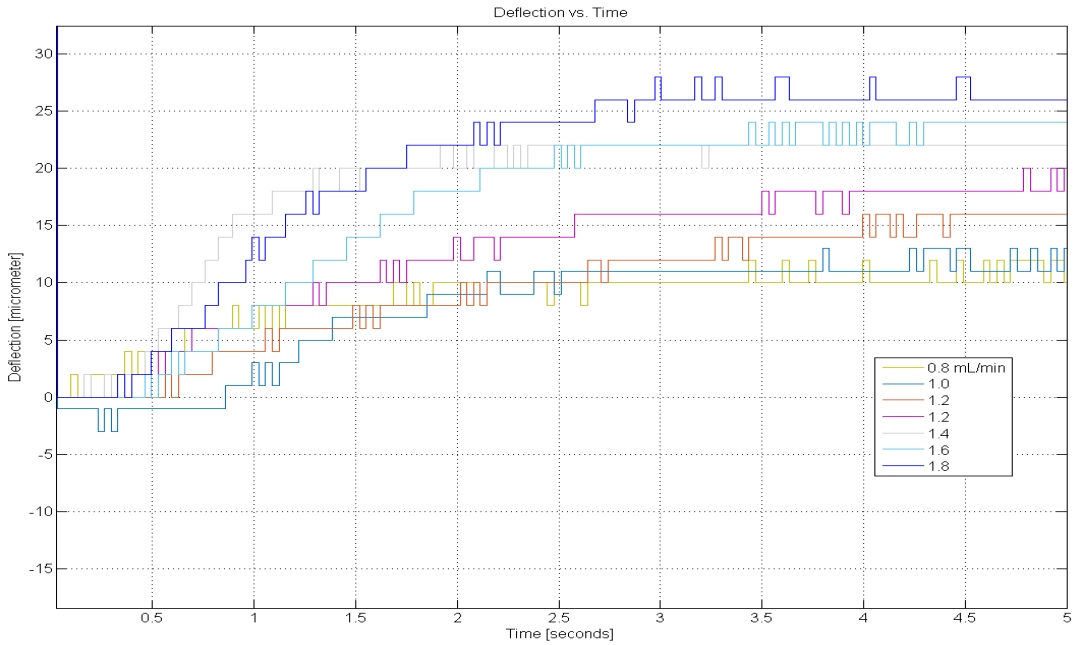
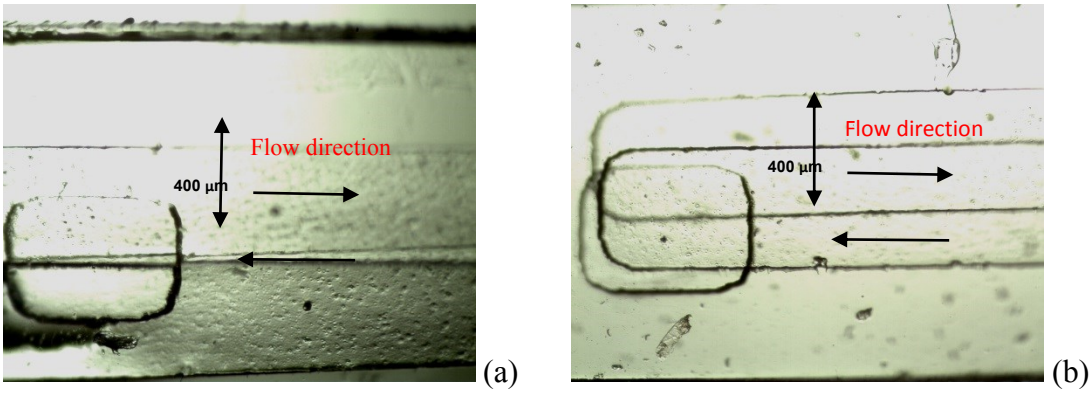


Figure A-3.Test results of the second SPMF^3



(a) (b)

Figure A-4. Two SPMF^3 platforms with different offset between their microchannels

Finally, the image processing measurement system sensitivity has a certain limitation which is the deflection measurement precision. As is was shown in the results, the SPMF^3 with the aforementioned dimensions is sensitive to flow rates of 0.2 ml/min and higher which is equal to microcantilever deflections of 5μm and above. However, for detecting lower flow forces and smaller deflections a more precise deflection measurement system is required.

A.1.2. Highly sensitive optical laser deflection measurement test setup

In order to improve the measurement resolution, an optical laser deflection measurement system was employed. Here, a geometrical design for the measurement system is depicted and its governing equation to obtain the microcantilever deflection is as follows (Figure A-5).

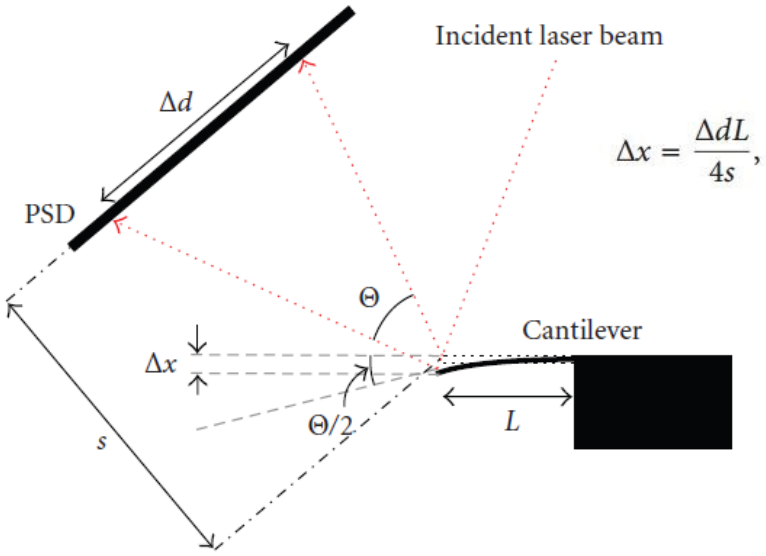


Figure A-5. Geometrical schematic laser deflection measurement system [119]

Where Δx is the cantilever deflection, Δd is the laser beam travel distance on the photo sensitive detector (PSD), L is the cantilever length and s is the distance between cantilever and PSD [119]. Although this geometrical problem is also solved with more details and

parameters in a 3D space [103] however, the current equation is enough for our calculation if all of the setup parameters such as PSD or laser angles remain identical among all of the experiment processes. Thus, the current equation results have been compared with extensive geometrical calculation which shows the same results in these two methods (Figure A-6).

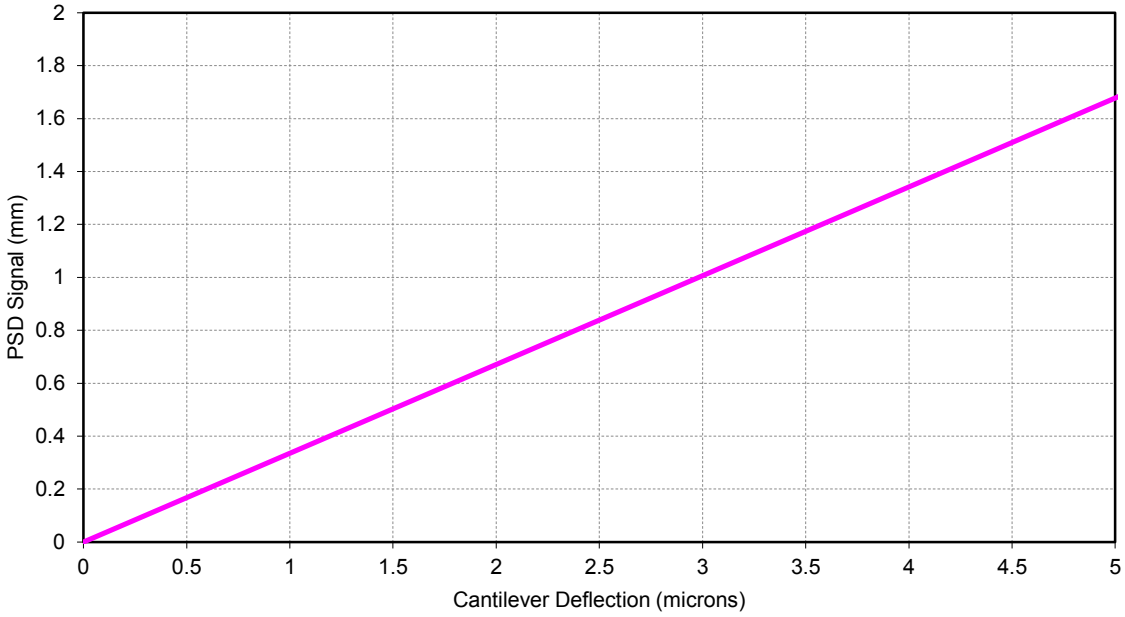


Figure A-6. Calibration chart of the laser deflection measurement setup

Following the main elements of the laser measurement setup which are laser with narrow beam (less than 1mm), photo sensitive detector (PSD) and fixtures to maintain the geometry of the measurement system are shown (Figure A-7).

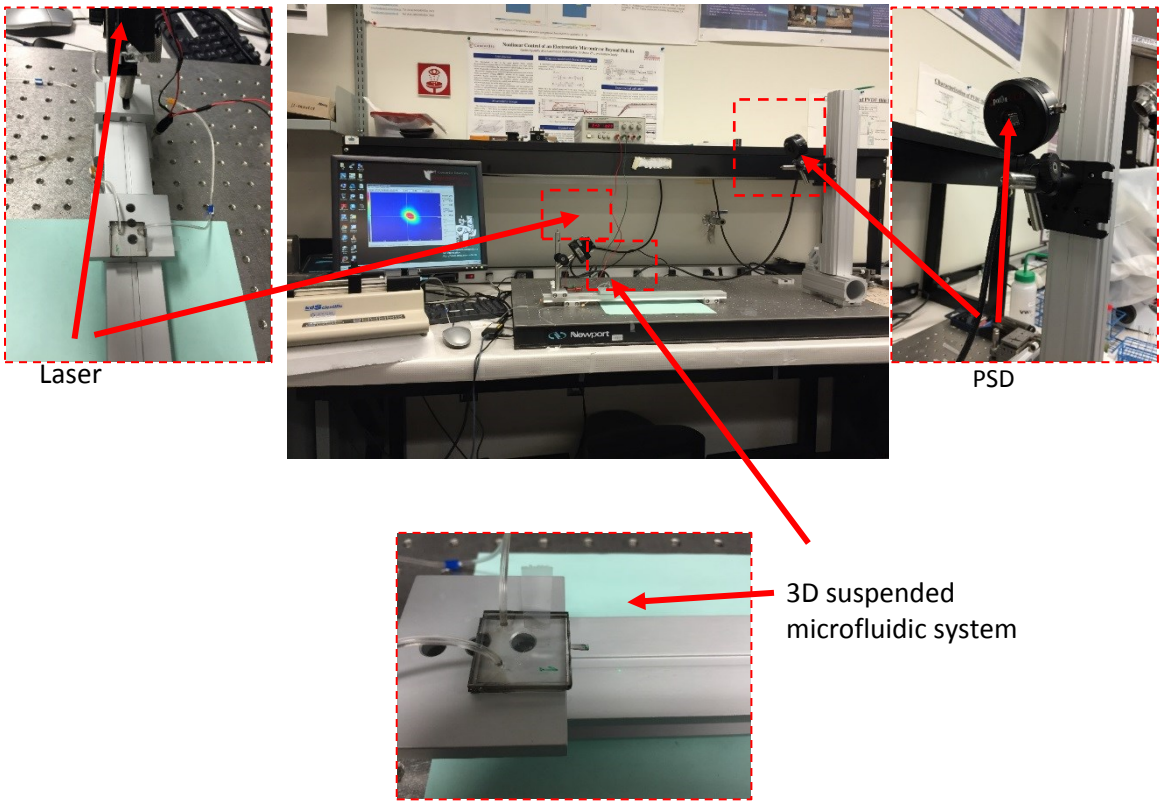
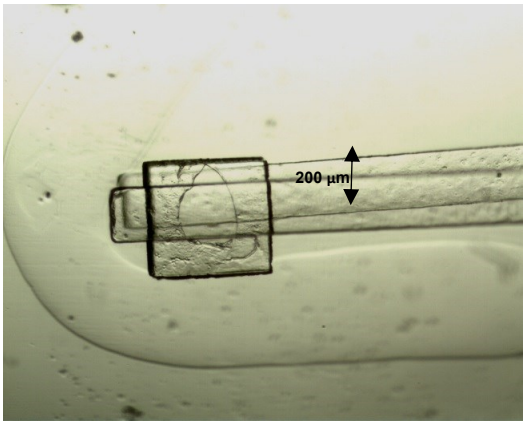
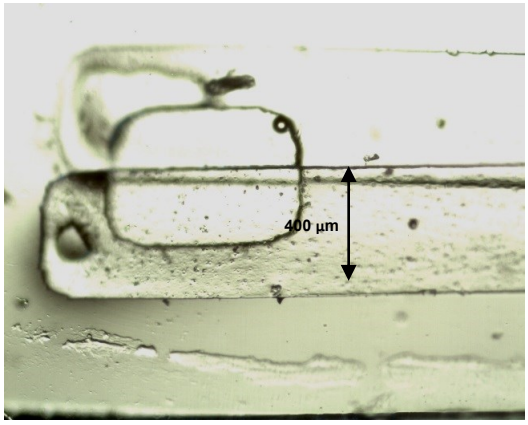


Figure A-7. Laser deflection measurement setup

A.1.3. Experimental results using the laser measurement setup

In this experiment, DI water with different flow rates was injected into the suspended microfluidics and microcantilever deflections were recorded using the laser beam deflection measurement setup. Two different suspended microfluidics were employed during these experiments which their microscopic picture is depicted here (Figure A-8).



(a)


(b)

Figure A-8. Two suspended microfluidics went under experiment a) SPMF^3 with 200 microchannel b) SPMF^3 with 400 microchannel

In order to access the sensitivity new measurement system, two different experiments were designed and performed. One is the flow rate measurement experiment and the second experiment is specifying fluid properties such as density and viscosity. These results were completely discussed in chapter two.

A) Micro Flow meter

A suspended microfluidics with 200µm microchannel was tested under DI water flow with different flow rates of 0, 10, 15 µl/min and the microcantilever deflections were monitored with the PSD as shown in Figure A-9. In this experiment, the syringe pump was switched on and off, shown in the graph, to extract the system response and the residual stress inside the microfluidics. Since this laser setup is very sensitive and can detect deflections of around 100 nm, some noise is also expected which is shown here. As it is shown in Figure A-9, there are two graphs, one is deflection signal with noises and another one is the filtered signal. However, using MATLAB and a low pass filter, all of the high frequency noises are canceled to improve the graph appearance.

A similar experiment was performed on another SPMF^3 with a 400µm microchannel. In this experiment, the syringe pump flow rate was switched between 0 to 20 µl/min and the result we depicted in Figure A-10. Some other experiments with different flow rate steps were carried and the results are depicted in Figure A-11. As it is seen here, now we can detect

flow rates of 10 µl/min and less which was impossible with the image processing setup to go less than 200 µl/min.

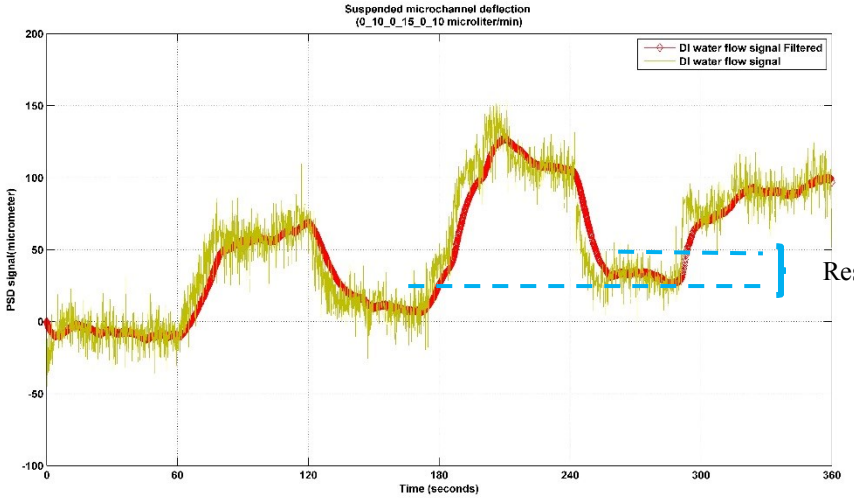


Figure A-9. Flow rate experiment on the SPMF^3 with 200µm microchannel

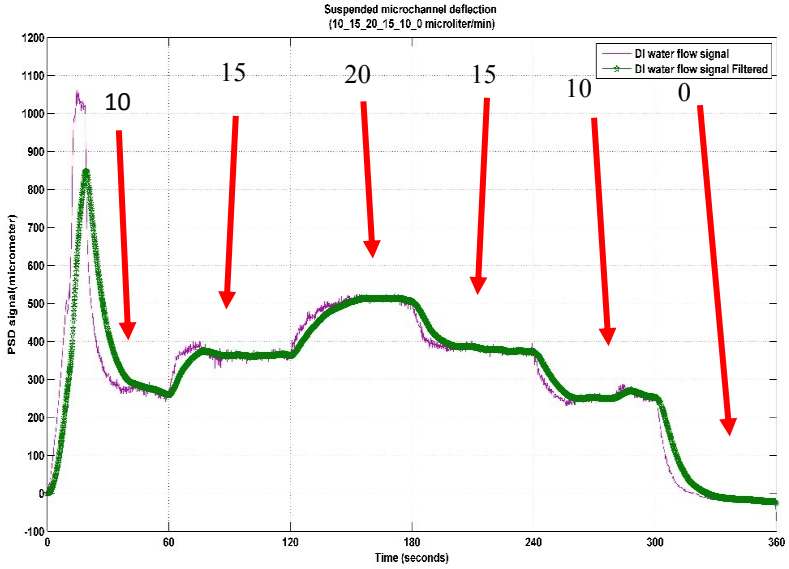


Figure A-10. Flow rate experiment of the SPMF^3 with 400µm microchannel; deflections under flow tares of 10, 15, 20, 10 and 0 µL/min

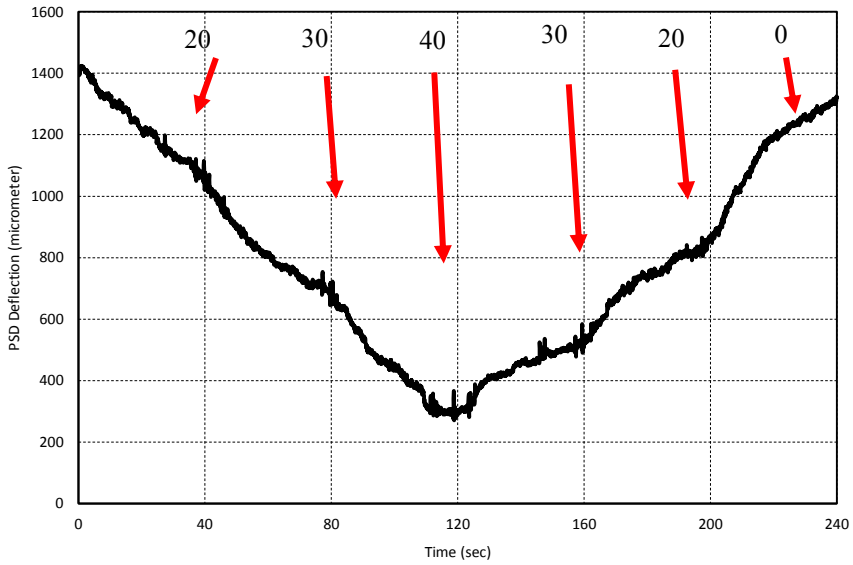


Figure A-11. Deflections under flow rates of 20, 30, 40, 30, 20 and 0 µL/min with a 200µm microfluidics

B) Micro-viscosity sensor

Real-time detection of variation in fluid properties is another application of the SPMF[3] for which two experiments were defined and performed successfully. In the first experiment, three different solutions with 0, 10 and 15wt% of salt in DI water was injected into the microfluidics using a syringe pump at a flow rate of 50 µl/min. In another experiment, milk with different fat concentrations of 0, 3.25, 10, 20 and 35wt% were employed. Following the fluid properties such as density and viscosity under different concentrations are gathered in Table A-1 which shows that any variation in fluid concentrations will affect both fluid density and viscosity due to the molecular level modifications discussed in chapter four.

Table A-1.Water- Salt solution and Milk-Fat fluid properties

Fluid	Concentration	Density (kg/m^3)	Viscosity (cP)
Salt wt %	0 %	999	1.002
	10 %	1070	1.193
	15 %	1110	1.350

Milk wt %	0 %	1033	3.594
	3.25 %	1030	4.192
	10 %	1025	4.797
	20 %	1012	6.598
	35 %	994	11.391

The SPMF^3 deflections and experimental results were presented and discussed in details in the fourth chapter. Here, some of the raw results of water-salt solution from the PSD signal is presented after noise cancellation in order to explain the data measurement process in more details. Figure A-12 shows the deflections of an SPMF^3 with 400µm microchannel under different DI-water and salt solutions. A similar experiment was performed on the SPMF^3 with 400µm microchannel and the results are shown in Figure A-13.

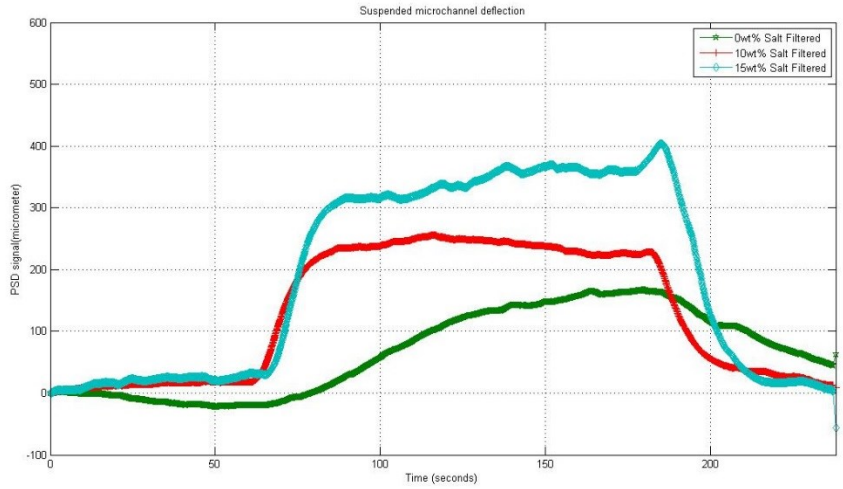


Figure A-12. Fluid detection experiment on the SPMF^3 with 200µm microchannel

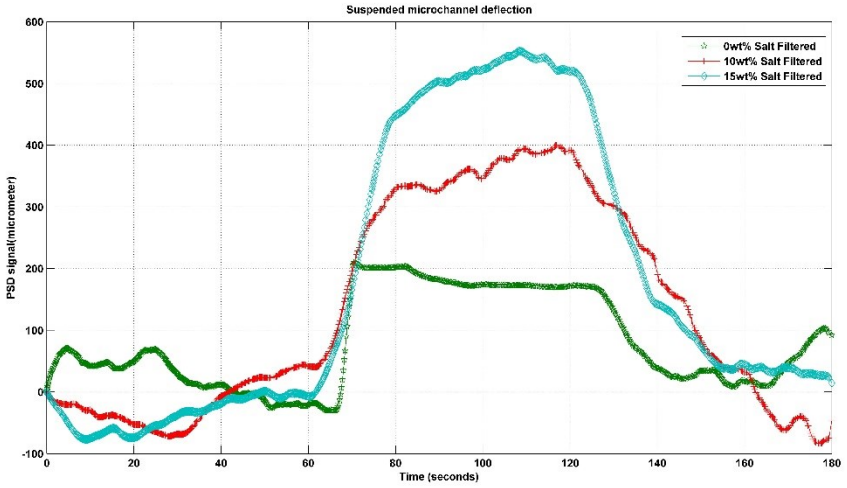


Figure A-13. Fluid detection experiment on the SPMF^3 with 400µm microchannel