An Integrated Microfluidic Platform for Chemical and Biological Sensing
Employing Polymer-coated Piezoelectric Microcantilevers

A Dissertation Presented

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Masoud Khabiry

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ABSTRACT

Microfluidic integrated systems offer several advantages compared to macroscopic techniques. The ability to handle small sample volumes, portability, and the low cost of the devices are the most significant of these advantages. Microfluidic systems have substantially advanced the landscape of chemical and biological research. Microfluidic platforms hold great promise in manipulation and examination of single cells, single molecules, sensor integration, chemical synthesis, biochemical assays, bioanalysis, and high-throughput screening. In addition, microfluidic systems have great potential for integration of Micro/Nano electromechanical systems (MEMS/NEMS) and microcantilever-based sensors.

In this work, an integrated microfluidic system with shear-protective regions that enables cell and particle immobilization, sensor integration, and nanoparticle synthesis is presented. Thus, a novel and simple microfluidic device for capturing small volume of cells by using sidewall microgroove containing channels and microposts is developed. The developed microfluidic system enabled the control of fluid flow and shear stress profiles. Furthermore, the shear stress variation and cell positioning in the sidewall microgrooves were investigated. Moreover, the histograms of cell locations in the microgrooves were provided and the most probable destination of the cells was presented. In the microfluidic device, further investigation on extracting cell information from image data was carried out. Hence, a cell segmentation technique was developed for cell counting and extracting the cell information from the microfluidic device.

This platform also has the capability of integration of polymer coated piezoelectric microcantilevers which can be functionalized for analyte detection. Piezoelectric microcantilever-based sensors provide less complex system, eliminate the need for external optics and optical alignments, operate under larger gap distances, consume less power and generate less heat. Furthermore, piezoelectric microcantilever-based sensors can operate in the self-sensing manner where a piezoelectric layer embedded in the structure of the microcantilevers can be used for both sensing and actuating purposes. Moreover, an excellent way of enhancing and broadening the applicability and functionality of piezoelectric microcantilever-based sensors is to coat them with a layer of sensitive polymer. The data obtained from analyte detection by polymer coated
piezoelectric microcantilever was presented. In addition, through a combined mathematical modeling the experimental findings were rationalized. Integration of microfluidic platform containing polymer coated piezoelectric microcantilever was presented. Utilization of three-dimensional (3D) printing method for developing of the microfluidic component of integrated system was discussed. Alternative physical set-up of the integrated microfluidic platform containing microcantilever array was presented.

Microfluidic devices are also able to rapidly mix reagents, and provide homogenous reaction environments. These features make them an ideal platform for nanoparticle synthesis. In this work, capability of nanoparticle synthesis by our microfluidic platform was also presented. In particular, flow focusing microfluidic technique for continuous synthesis of nanoparticles was examined.

There are some improvements that can be pursued for further investigation on the presented integrated platform such as using different shapes and angles of sidewall microgrooves. Overall, it is possible to use this simple and adaptable platform for a sensitive detection of a wide range of analytes.
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DEDICATION

This dissertation is dedicated to my wife, my daughter, my father, and in the loving memory of my mother.
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CHAPTER 1

INTRODUCTION

1.1 MICROFLUIDICS

Microfluidic devices allow precise manipulation of nanoliter volumes of liquids and have recently led the rapid advancement of experimental methods in chemical and biological sciences [1]. Microfluidic devices offers the possibility of controlling of fluid flow, generating stable concentration gradients, and regulating cell-soluble factor interaction in a temporal and spatial manner [1-5]. Moreover, microfluidic methods are particularly well compatible with biological materials such as cells and allow researchers to precisely control the cellular environment in culture and to miniaturize assays [6-8]. This is especially true for device materials such as poly(dimethylsiloxane) (PDMS), which is commonly used to fabricate active and passive fluidic channels [6-8]. Microfluidic devices can also be used to control the cellular microenvironment by shear stress on cells as well as regulating the temporal and spatial presentation of soluble factors [9]. Furthermore, the microfluidic devices enable the control of cell docking and immobilization in a well-defined microenvironment, features necessary for cell-based screening applications and cell-based biosensing [10-15].

To immobilize cells within microfluidic channels using microstructures, a number of approaches such as cup-shaped high-density hydrodynamic cell isolation microfluidic device, microfluidic cell pairing device have been developed [16-20]. Although cup-shaped microstructure in the
microfluidic channel enables the capture of individual single cells within both front-side larger cup and back-side smaller cup, a complex three-step cell loading process is required. Moreover, a multilayer microfluidic device with permeable polymer barriers for capture and transport of cells with microvalves should be developed that requires alignment and complex fabrication process [21]. It is also possible to immobilize cells within microfluidic channels, by using methods such as encapsulation within photocrosslinkable polymers, adhesion to patterned proteins, and protein coatings [16-18]. However, all of these approaches require complex and costly surface treatment of the microchannel prior to fabrication.

An alternative approach to capture cells within fluidic systems is through the use of grooved substrates [9, 12]. These microgrooved regions located at the bottom of the substrate provided shear-protective regions and regulated micro-circulation, resulting in cell docking and positioning; however, these approaches have some limitations, such as cells were attached on the bottom substrates and it might be difficult to control the docking of small number of cells. Moreover, these approaches requires alignment and complex fabrication process. Thus, to overcome these challenges, sidewall microgroove containing channels to regulate cell docking and positioning is considered. By using the sidewall microgrooves in the microchannels, the capture of small number of cells within microfluidic device is enabled, showing more control over cell docking and positioning.

Microfluidic systems have also been used to produce nanoparticles. Microfluidics and microreactor based systems have enabled the synthesis of nanoparticles with narrower size distributions, and improved batch-to-batch reproducibility [22, 23]. Batch (Bulk) synthesis of nanoparticles tends to suffer from irreproducibility of size, size distribution, and quality of the nanoparticles from batch to batch [24]. It is also difficult to implement fast screening and
optimization of the synthesis conditions in batch, and there are challenges in scaling batch procedures up to quantities needed for device development and optimization [24]. Some key advantages of microfluidics include simplicity and reproducibility of device fabrication and potentially lower cost of materials due to the ability to handle small volumes [22]. These advantages make the use of microfluidics ideal for the development of a platform that enables rapid synthesis and optimization of nanoparticles [22, 23].

1.2 PIEZOELECTRIC MICROCANTILEVER BASED SENSOR

There has been a growing effort and interest in the development of micro/nanomechanical cantilever-based sensors in recent years [25, 26, 27]. In the past decade, microcantilever-based sensors have emerged as an extremely high sensitive technology for the detection of chemical analytes and biomolecules [25].

Microcantilever-based sensors can be operated in static or dynamic mode. In static mode, the deflection of microcantilever changes by adsorption of analyte [28]. This deflection can be measured and correlated to the amount and type of adsorbed substance. In the dynamic mode, the resonance frequency of microcantilever reduces due to additional mass loading by adsorbed analyte [29, 30]. The sensitivity of the microcantilever-based sensors in this mode of operation can approach up to a sub-picogram resolution [29, 30]. The versatility, sensitivity, and superior detection limits of Microcantilever-based sensors over other detection methods such as surface acoustic wave (SAW), flexural plate wave oscillators (FPW), acoustic plate mode (APM) devices, and quartz crystal microbalances (QCM) have been shown in the literature [31, 32].

Microcantilever-based systems are capable of real-time, multiplexed detection of analytes and biomarkers in a very small volumes of samples [33]. Current microfabrication techniques have
enabled the integration of electronic readout and sample introduction into a single unit thereby reducing cost, decreasing the device size, and the detection time can be significantly shortened [33-35].

Important advances have been made to use piezoresistive Microcantilever-based sensors in environmental applications, where these microcantilever sensors detect based on static bending [26]. However, the piezoresistive effect of these microcantilevers consumes milliwatts of power to make measurements thus generating unwanted local heat [26]. A better way to address these challenges is to use piezoelectric microcantilevers [25, 27]. Piezoelectric microcantilevers can serve as sensor and actuator (self sensing mode) with low power consumption [25-28].

1.3 STRUCTURE OF THE DISSERTATION

The subsequent chapters of this dissertation are organized as following. In Chapter 2, a novel and simple microfluidic platform for capturing small volume of cells by using sidewall microgroove containing channels and microposts is described and presented. Moreover, both numerical and experimental investigations are discussed.

Chapter 3 presents a unique polymer-coated piezoelectrically (ZnO) driven microcantilever for chemical analyte detection. Furthermore, the experimental evidence and theoretical rational of analyte detection by our developed system have been presented. In addition, integration of microfluidic platform containing polymer coated piezoelectric microcantilever have been demonstrated. In Chapter 3, the utilization of three-dimensional (3D) printing method for creating microfluidic component of proposed integrated platform was discussed.
In the proposed microfluidic device, further investigation on extracting cell information from image data was needed. Hence, in Chapter 4, a cell segmentation technique is presented and used for cell counting and extracting cell information in a microgroove based microfluidic device.

In Chapter 5, capability of nanoparticle synthesis by our microfluidic platform have been demonstrated. The concept of flow focusing microfluidic device have been discussed. In addition, chapter five discusses the exploratory portion of our nanoparticle generation demonstrating the synthesis of two different nanoparticles, namely, gold and liposomes by using flow focusing microfluidic technique.

Each of Chapters 2, 3, 4, and 5 includes an introduction related to the numerical and experimental approach or related background, a materials and method section or experimental setup section, followed by results and discussion, as well as a summary section. Finally, conclusions and suggestions for future directions have been summarized in Chapter 6 of this dissertation.
A MICROFLUIDIC PLATFORM CONTAINING SIDEWALL MICROGROOVES FOR CELL POSITIONING AND TRAPPING

In this chapter, a simple microfluidic platform is proposed for capturing small volumes of cells by using sidewall microgrooves. The cell docking patterns in the sidewall microgroove containing channels are also studied. Both numerical and experimental investigations are performed within sidewall microgroove containing channels with three different widths (i.e., 50, 100, and 200 µm). It is observed that sidewall microgroove containing channels play an important role in regulating cell positioning and patterning. Obtained results revealed that 10 to 14 cells were positioned inside sidewall channels with 200 µm in width, 2 to 5 cells were positioned within 100 µm wide, in contrast, and 1 to 2 individual cells were docked within sidewall channel with 50 µm in width.

Particle modeling showed the prediction of cell positioning within sidewall microgrooves. The positions of cells docked within microgroove containing channels were also quantified. Furthermore, the shear stress variation and cell positioning in the sidewall microgrooves were correlated. Therefore, this sidewall microgroove containing channels could be potentially useful for regulating cell positioning and patterning on two-dimensional surface, three-dimensional microenvironments, and high-throughput screening. Cell patterning and positioning are of great importance in many biological applications such as drug screening and cell-based biosensing.

*Most or part of the writeup in this chapter might have directly come from authors’ publication (M. Khabiry, N. Jalili, 2015, Nanobiomedicine [289])
2.1 INTRODUCTION

Microfluidic platforms hold great promise for biochemical synthesis, high-throughput drug screening, and cell-based biological assay [1-5]. Microfluidic devices offers the possibility of controlling of fluid flow, generating stable concentration gradients, and regulating cell-soluble factor interaction in a temporal and spatial manner [1-5]. The poly(dimethylsiloxane) (PDMS)-based microfluidic devices offer a number of advantages, such as low cost, short reaction time, high-throughput analysis, and real-time monitoring of biological processes [1,8,15,36-43]. Furthermore, the microfluidic devices enable the control of cell docking and immobilization in a well-defined microenvironment, features necessary for cell-based screening applications [9-14]. Moreover, cell patterning and positioning are of great importance in many biological applications such as drug screening and cell-based biosensing [15].

It has been shown that the microfluidic devices containing shear-protective microgrooved regions located at the bottom of the substrate have the ability to control cell positioning [9-12]. The previous microgrooved regions located at the bottom of the channels provided shear-protective regions and regulated micro-circulation, resulting in cell docking and positioning; however, these approaches have some limitations, such as cells were attached on the bottom substrates and it might be difficult to control the docking of small number of cells. To overcome these challenges, sidewall microgroove containing channels to regulate cell docking and positioning were considered. By using the sidewall microgrooves in the microchannels, the capture of small number of cells within microfluidic device is enabled, showing more control over cell docking and positioning. Furthermore, it may be possible to co-culture different cell types in the sidewall microgrooved channels. It has been shown that the microfluidic system containing high-quality and small volume of cells is required for studying quantitative system biology [19-20]. For
example, cup-shaped high-density hydrodynamic cell isolation microfluidic device has been previously developed [19]. Individual cells were docked within cup-shaped microstructures and single cell enzymatic kinetics was analyzed. Two-layer cup-shaped arrays allowed for the fluidic streamlines for the cell trapping. When one cell was occupied within a cup-shaped array, flow was diverted and then another cell was trapped within a neighboring cup-shaped array. Furthermore, microfluidic cell pairing device has been developed to study electrical fusion analysis [20]. Two cell types were captured and paired in two cup-shaped cell isolation microfluidic device containing larger capture cup and smaller back-side capture cup. Although this microfluidic channel enables the capture of individual single cells within both front-side larger cup and back-side smaller cup, a complex three-step cell loading steps are required. Moreover, a multilayer microfluidic device with permeable polymer barriers for capture and transport of cells with microvalves was developed that requires alignment and complex fabrication process [21]. In contrast, our proposed microfluidic platform provides significant advantages over these methods. It: (i) is very simple; (ii) is one layer, (iii) provides a platform for capturing small number of cells; (iv) uses one-step microfabrication process which does not require any alignment between bottom substrate and microfluidic channel; and (v) does allow for high-density microscopic analysis.

In this chapter, a microfluidic device containing sidewall microgrooves that enables trapping and positioning of cells in a controlled manner is developed. Furthermore, cell positioning in sidewall microgrooves is analyzed. The effect of the cell docking and positioning on the sidewall microgroove containing channels is also investigated. Computational simulations provided estimates of particle tracing patterns which were accurate proxies for cell positioning. Computational modeling is compared to experimental results of cell docking within sidewall microgrooved channels. The particle trajectory was also predicted in sidewall microgrooves
containing square microgeometry. Both numerical and experimental results are presented to demonstrate that the proposed microfluidic device containing sidewall grooves in the microchannel could be a potentially useful tool for studying the docking and positioning of small number of cells down to 1 to 2 individual single cells.

2.2 MATERIALS AND METHODS

Fabrication of the microfluidic device containing sidewall microgrooves

Microfluidic devices with sidewall microgrooves were fabricated by using the photolithography technique that has been previously developed [17, 44-46], see Figure 2.1. The silicon master mold was made by using a negative photoresist (SU-8 2050, Microchem, MA). For making sidewall microgroove patterns with 80 µm thick, SU-8 2050 was spin-coated using 1,500 rpm for 60 sec, baked for 8 min and 25 min at 65°C and 95°C, respectively and exposed to UV for 3 min. After UV exposure, photoresist-patterned silicon master was post-baked for 1 min and 8 min at 65°C and 95°C, respectively. The negative replica of the microfluidic channel was molded in poly(dimethylsiloxane) (PDMS) (Sylgard 184 Silicon elastomer, Dow Corning, MI). The PDMS prepolymer mixed with silicone elastomer and curing agent (10:1) was poured on the master and cured at 70°C for 2 hours. PDMS molds were removed from the photoresist-patterned master. An inlet and outlet of microfluidic channel were punched by sharp punchers for the cell seeding and medium perfusion. The sidewall microgroove containing channel and bottom PDMS substrate were irreversibly bonded by using oxygen plasma (5 min at 30W, Harrick Scientific, NY). Sidewall microgrooves in the microchannels were perpendicularly placed to the fluidic flow direction in a microfluidic device.
Cell docking in a microfluidic device

NIH 3T3 mouse fibroblasts were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen, CA) containing 10% Fetal Bovine Serum (FBS, Invitrogen) and 1% penicillin/streptomycin (Invitrogen, CA). To seed the cells into the microfluidic sidewall channel, the cells were trypsinized and dissociated with culture medium. A counting chamber, also known as hemocytometer, was used to obtain the cell density. The cells were seeded in a microfluidic device through a cell inlet port at the cell density of \(6 \times 10^6\) cells/mL. After 20 minutes of cell seeding, medium was infused by using a syringe pump at a flow rate of 5 µL/min. The medium was pumped to the inlet port of the microfluidic device (the obtained flow direction was from left to right as it is illustrated by an arrow in Figure 2.2C). Cell docking in the sidewall microgrooves containing channels with three different widths of sidewall microgrooves (i.e. 50, 100, and 200 µm in widths) was analyzed.

Image analysis for cell docking and retention

Cell images were obtained by using an inverted microscope (Nikon TE 2000-U, USA). To analyze cell docking within sidewall microgrooves in the microchannel, cell numbers and their location through image analysis were obtained. The average cell size in the microgrooves was quantified by ImageJ software. The size of loaded 3T3 fibroblasts cells was on average 10 µm. The experiments were performed with different microgroove sizes three times in a microfluidic device. Statistical analysis was performed by using the student t-test.

Numerical Simulations

Computational Fluid Dynamic (CFD) modeling was used to simulate the fluid flow behavior in the sidewall microgroove containing channels using the finite element method (COMSOL 3.4, Burlington, MA). There exists ample literature on CFD, finite volume, finite element methods [47-]
49], and fluid flow in cavities [50-57]. The laminar incompressible flow in a square cavity whose
top wall moves with a uniform velocity in its own plane has served over and over again as a model
problem for testing and evaluating numerical techniques [56, 60-65]. The detailed discussion of
mathematical modeling of two dimensional lid driven cavity flow is discussed in Appendix A.
To estimate the cell penetration into the sidewall microgrooves, 3D numerical simulation of
experimental setup was performed. An experimental, analytical, and computational study of the
removal of submicrometer particles at different depths inside the trench (cavity) is already
investigated [66, 67]. Furthermore, the effects of frequency, temperature, and power density in
particle removal in megasonic and ultrasonic cleaning were studied [68]. However, here our focus
is to investigate on cell penetration and shear stress variation in the sidewall microgrooves. In our
modeling, unstructured mesh generation method was used for constructing the 3D mesh domain.
Our fluid modeling is based on incompressible Navier–Stokes equations [58, 59] with Stoke's
hypothesis assumption in conservation form for an arbitrary geometry. The governing equations
can be written as follows:
Continuity Equation:
\[
\frac{\partial \rho}{\partial t} + \nabla \cdot \left( \rho \mathbf{V} \right) = 0
\]  
(2.1)
Momentum Equation:
\[
\rho \left( \frac{\partial \mathbf{V}}{\partial t} + \mathbf{V} \cdot \nabla \mathbf{V} \right) = -\nabla p + \mu \nabla^2 \mathbf{V}
\]  
(2.2)
where \( \mathbf{V} \) represents velocity (m/s), \( p \) pressure (Pa), \( \rho \) density (kg/m\(^3\)), \( \mu \) dynamic viscosity of
fluid (Pa. Sec), and \( t \) time (sec). The properties of fluid (medium) in our modeling are considered
to be the same as water; which implies the density of 1000 (kg/m\(^3\)) and dynamic viscosity of 0.001
(Pa. Sec).
For our numerical modeling, the boundary condition at the walls and the bottom of the microgrooves are set up to be no-slip boundary conditions. The specified velocity condition is applied for the inflow boundary condition (Dirichlet boundary condition). Furthermore, the criteria for convergence (RMS residual) are considered to be equal to $10^{-6}$.

### 2.3 RESULTS AND DISCUSSIONS

**Sidewall microgroove containing channels**

A PDMS-based microfluidic device with sidewall microgrooves was developed to regulate and control cell positioning and docking (Figure 2.1). This microfluidic device mainly consists of sidewall microgrooves (50×50, 100×100, and 200×200 μm) and posts (125, 250, and 375 μm radius) that enables control of flow velocity and shear stress profiles. Three types of microchannels (500, 1000, 1500 μm widths) were fabricated. A microchannel with a 500 μm width has a 250 μm post diameter, microchannel with a 1000 μm width has a 500 μm post diameter, and a microchannel with a 1500 μm width has a 750 μm diameter of post. As shown in Figure 2.1B, our fluidic channel containing sidewall microgrooves were irreversibly bonded to a PDMS substrate.

To analyze cell positioning within sidewall microgrooves, cells were seeded into a microfluidic device through a cell inlet port and medium was subsequently infused by using a syringe pump. This microfluidic device has several advantages over previous cell docking microfluidic platforms, because it can regulate the docking of small number of cells down to 1 to 2 individual single cells in comparison with the earlier studies [9, 12]. The one-step microfabrication process that was used in this paper did not require any alignment between microgrooves and microfluidic channel layer whereas it is an essential part of other approaches [19, 21].
Sidewall microgrooves were fabricated in the channels to analyze cell docking behavior without any gravity effect that has been usually generated within bottom microgrooved channels [9, 12]. It is shown that the cell docking in bottom microgrooved channels is significantly regulated by gravity and shear stress profiles [9, 12]. Thus, it is not easy to identify which parameter is more important to regulate and control cell docking and positioning. To address this issue, sidewall microgrooves were developed in the microchannels. Additionally, to better understand the effect of geometrical factors, four different parameters were varied that include post radius \( (R_p: 125, 250, 375 \, \mu m) \), channel width \( (W_c: 500, 1000, 1500 \, \mu m) \), microgroove length \( (L_g: 50, 100, \text{and} \, 200 \, \mu m) \), and microgroove depth \( (W_g: 50, 100, \text{and} \, 200 \, \mu m) \). The geometrical parameters involved are shown in Figure 2.1B. These four spatial variables were also scaled by the microgroove width \( W_g \) which is equal to the microgrooves length \( L_g \) in our studies. Moreover, two dimensionless ratios were defined \( W_c^* = W_c / W_g \), and \( R_p^* = R_p / W_g \). As a result, the number of geometrical factors involved were reduced to two dimensionless ratios. The obtained values of \( R_p^* \) corresponding to the microfabricated microfluidic devices are 0.625, 1.25, and 1.875, respectively. Both experimentally and theoretically, the effect of these different parameters for cell docking and positioning within sidewall microgroove containing channels were evaluated.

**Cell positioning within sidewall microgrooves**

Cell docking and positioning were analyzed within a microfluidic channel containing sidewall microgrooves \( (50, 100, \text{and} \, 200 \, \mu m \text{ in widths}) \) (Figure 2.2). Each sidewall microgroove was placed as half spacing as their widths within a microfluidic channel. Figure 2.2A-C represents the cell distribution within the sidewall microgrooves. Through an image analysis approach, the
number of cells and their position within sidewall microgroove channels were obtained. As discussed earlier, the flow direction is presented in Figure 2.2C by an arrow and it is from left to right. Hence, the center, upstream, and downstream of sidewall microgrooves was classified based on the flow direction and the position of the post. Cell docking analysis showed that cell docking was significantly regulated by the geometry (i.e. groove width) of sidewall microgrooves. Figure 2.3A, B, C provides an easy comparison of cell counts for three different sidewall microgrooves.
(50, 100, and 200 μm in widths). It was found that different numbers of cells were docked within three sidewall microgrooves. It was revealed that 2 to 5 cells were positioned within 100 μm wide sidewall microgrooves, while 10 to 14 cells were docked within 200 μm wide sidewall microgrooves in a microfluidic device with a 500 μm channel width. However, only a few cells (1 to 2) were docked within 50 μm wide sidewall grooves.

Cell docking results demonstrated that the number of cell docking within larger sidewall microgrooves (200 μm in width) is much higher than that of cell docking within smaller sidewall microgrooves (50 μm in width). Significant differences between number of cells docked in the microfluidic device with 500 μm and 1500 μm channel widths were not observed. It can be related to the small size of the sidewall groove relative to the width of the channel itself. Not much difference of cell docking among sidewall microgrooves of upstream, center, and downstream were observed. This can be explained because of the fact that the post is located far away from the sidewall microgrooves. If the distance between a post and sidewall microgroove was short, the number of cell docking at the center of sidewall grooves might be higher as compared to upstream and downstream of sidewall microgrooves. To confirm this hypothesis, fluidic flow and shear stress profiles were simulated. The obtained results for simulation is discussed in the theoretical modeling section. Generally, it was observed that cells were positioned and located at the center of sidewall microgrooves. Therefore, the obtained result in sidewall microgrooves should prove useful to co-culture different cell types.

To support the experimental data, cell docking and positioning were analyzed by using histograms. Figure 2.4 represents the two dimensional projection of 3D histogram for different microgroove sizes and channel widths. For comparison purposes, the length and width of all microgrooves for each channel width 500, 1000, and 1500 μm were normalized in Figure 2.4. The aforementioned
histogram verifies the distribution probability of cells inside the sidewall microgrooves is higher in the highlighted regions. It is also observed that the histogram distribution has tendency toward the central region of the sidewall microgrooves, and the probability of the cell docking is higher in the middle of the microgrooves. This observation indicates that cells will be docked within designated shear-protective sidewall microgrooves. Consequently, the cell dockings and positioning can be regulated and controlled by this approach. It was noted that those cells that were not docked within shear-protective region were removed by the medium perfusion.

**Figure 2.2** Cell docking in sidewall grooves. (A-C) Phase contrast images of cell docking within sidewall grooves ($W_g$=50, 100, and 200 µm) in a microchannel ($W_c$=500, 1000, and 1500 µm). Scale bars: 100 µm. ([289] with permission)
Micropost design considerations
One of the distinct features of our microfluidic device is based on the incorporation of the microposts. These posts are aligned in the middle of the microchannels as shown in Figure 2.1A. The microposts play an important role in flow diversion. Moreover, these microposts facilitate the changing of the streamline patterns and velocity contours. A schematic presentation of flow diversion around the post is shown in Figure 2.5A. This diversion in the fluid flow should prove useful in case of delivering different drugs to the cells immobilized in the upper microgrooves vs. those residing in the lower microgrooves. The effect of incorporation of micropost on 3D particle simulation within the sidewall microgroove with and without micropost is illustrated in Figure 2.5B, C. In addition, the change in the velocity distributions within the sidewall microgroove with and without micropost is shown in Figure 2.5D, E. Furthermore, the effect of inclusion of micropost and changing its diameter on the streamline distribution is demonstrated in Figure 2.6A, B, and C. It

Figure 2.3 (A-C) Quantitative analysis of cell docking within sidewall grooves (W_g=50,100, and 200 µm) at upstream, center and downstream in a microchannel (W_c=500, 1000, and 1500 µm). ([289] with permission)
was noted that by inclusion of micropost the streamlines are getting closer together underneath the micropost. This streamline pattern change causes an increase in the fluid velocity below the micropost region while keeping the velocity in the microgroove area very low. Furthermore, through the particle simulation it was noted that inclusion of the micropost facilitate better particle penetration in the sidewall grooves.

![Diagram showing cell distribution in sidewall grooves](image)

**Figure 2.4** Cell distribution in sidewall grooves: Projection of 3D histogram in horizontal plane of cell distribution within side microgrooves (Wg=50, 100, and 200 µm) in a microchannel (Wc=500, 1000, and 1500 µm); top left: illustration of the projected region in a sidewall groove. ([289] with permission)
Theoretical modeling for the cell position

A variety of numerical experiments for sidewall microgrooves were investigated. As mentioned earlier, to consider all the fabricated microfluidic devices, different geometry and channel sizes were simulated. Hence, three different microgroove sizes were considered. It is started from the 200x200, continued by the size of 100x100, and then the size of 50x50. The three dimensional modeling of the sidewall microgrooves is considered since in our case studies the depth

Figure 2.5 (A) Schematic presentation of flow diversion around a micropost (B,C) 3D particle simulation within the sidewall microgroove with micropost and without micropost. (D,E) Velocity distributions within the sidewall microgroove with micropost and without micropost. (M. Khabiry, N. Jalili, 2015, Nanobiomedicine, with permission)
(perpendicular to the screen) to the height ratio of the microgrooves was not above one. Therefore, it will not be well justified to use the two dimensional modeling for prediction of flow pattern and streamlines in our solution domains. In the modeling the maximum Reynolds number was $Re_{\text{max}}=0.375$. This range of Reynolds number is in the limit of laminar flow or more precisely creeping flow $Re<1$. Hence, the obtained experimental flow regime is in agreement with the presented numerical modeling and consistent with the assumptions made for analytical solution.

The shear stress variation inside the groove has been shown in Figure 2.7. Different sections in the groove have been considered. The shear stress variation is shown for different groove sizes and different channel widths. In the groove itself, three horizontal (Upper, Middle, Lower part) are shown by letters a, b, and c (Figure 2.7). It was observed that the shear stress is one order of magnitude lower in the 50x50 groove size in comparison with 100x100 and 200x200
Figure 2.7 Shear stress distribution in sidewall microgrooves for three different geometry setup with (Wg=50, 100, and 200 µm) in a microchannel (Wc=500, 1000, and 1500 µm); bottom left: illustration of the specified paths which shear stress distribution shown along the horizontal lines a-a, b-b, and c-c . ); bottom right: illustration of geometric dimensions. ([289] with permission)
grooves. Moreover, there is not much variation from a-a, b-b, and c-c sections of the 50x50 groove size. It was also noted that there are two more peaks in the shear stress profile (c-c section) of the 50x50 groove size in comparison with the others. This observation can be explained by considering the fact that there is a combined corner and wall effect on this small region and also velocity is extremely lower in this region. In contrast, the larger the groove size gets 100x100 and 200x200, as shown in Figure 2.7, the shear stress is higher in the (a-a) section or upper part of microgroove,

**Figure 2.8** Shear stress distribution in sidewall microgrooves. (A) Effect of variation of channel width for three different pole radius $R_p^*=0.625$, 1.25, 1.875 along the horizontal line a-a (The schematic of the specified horizontal path a-a), ([289] with permission)
whereas it decrease in the middle and especially lower part of the groove. This explains the reason most of the cells accumulate in the middle section of the groove. It can be concluded that there is a threshold window where the cells prefer to stay in that region. It can also be seen that the experimental observations is in good agreements with the numerical simulations. The cells lie in the predicted region by the numerical modeling and it can be correlated to the histogram of cell positions which are provided in Figure 2.4.

Figure 2.9 Cell penetration (z) in sidewall microgrooves for three different geometry setup with (Wg=50, 100, and 200 µm) in a microchannel (Wc=500, 1000, and 1500 µm); bottom right: illustration of the specified coordinates mapped in side wall groove and cell penetration (z) . (M. Khabiry, N. Jalili, 2015, Nanobiomedicine, with permission)
To study the effect of varying the channel width on the shear stress variation inside the microgroove, the micropost radius was kept constant and width of the channel was changed. Simulations were run for the representative values of $R_p^*$ (0.625, 1.25, and 1.875) which were corresponding to the microfabricated microfluidic devices. For each case study, the microchannel width $W_c^*$ was chosen between 1.25 to 5 for covering all the corresponding microfabricated geometries. The obtained results for the upper region of the microgroove (a-a section) are shown in Figure 2.8. The shear stress magnitude decreases by increasing the channel width, and it is applicable for all three microfabricated channel widths. As expected, the wider the microchannel becomes the velocity and shear stress values will decrease given all the parameters constant. Furthermore, the obtained numerical results correlate the effect of micropost size in the shear stress distribution and cell positioning. Therefore, microposts can play a role as a geometrical control over the cell positioning in the sidewall microgrooves.

To further understand the effect of variation of the width of microgroove on cell penetration, a set of numerical simulations were performed for three different geometry setup with a microchannel width of $W_c$ (250, 500, and 750 µm). Cell penetration defined by $Z$ is shown in Figure 2.9. In all the cases it was assumed the micropost center and the groove are aligned perfectly, and microgroove length were normalized. Generally, it was noted that by increasing the microgroove size the cell penetration increases. These graphs are in agreement with the obtained values in the previous graphs and data. To expand our numerical simulations, a variety of numerical experiments for sidewall microgrooves with different dimensions than those shown in Figures 2.7, 2.8 and 2.9 are investigated and obtained results are presented here. The shear stress variation inside the microgroove is shown in Figures 2.10 and 2.11. Different sections in the groove have been considered and normalized by groove width (represented by star sign). The representative
values of $R_p^*$ (0.625, 0.875, 1.125, and 1.375) and microchannel width $W_c^*$ (3.3, 2.8, 2.3, and 1.8) which were different than the experimental microfluidic devices were chosen. The shear stress distribution for these test cases are shown in Figures 2.10 and 2.11, respectively. It was observed from Figures 2.10 and 2.11 that the shear stress is higher in the (a-a) section or upper part of the microgroove, whereas it decrease in the middle (b-b) and especially lower (c-c) part of the groove. This is consistent with the results obtained in Figures 2.7 and 2.8. Furthermore, effect of variation of width in the cell penetration for three different geometry setup with $R_p^*$=0.625, 1.25, and 1.875. within the sidewall microgroove is presented in Figure 2.12. In these simulations, the values of the microchannel width $W_c^*$ were varied from 1.25 to 4.3. It was observed that by keeping the micropost radius constant and increasing the microgroove width the cell penetration is deeper. The obtained graphs reveals the similar behavior presented in Figure 2.9.

2.4 CHAPTER SUMMARY

In this chapter, a unique and simple microfluidic platform for capturing small volume of cells by using sidewall microgroove containing channels and microposts was developed. It was demonstrated that the micropost size has an effect on the shear stress distribution inside the microgrooves. It was also observed that microgroove size plays a key role in cell capturing and cell positioning. In addition, the numerical modeling to predict cell positioning inside the microgroove is presented. The effect of channel width variation on the cell penetration is also investigated. Furthermore, the histograms of cell locations in the microgrooves were provided and the most probable destination of the cells was shown. Sidewall microgrooves containing channels provide a platform for cell positioning, shear protected area for cell study, and easily observable by a microscope. Hence, this simple yet adaptable microfluidic device should be useful for high-throughput screening, cell-based biological assay, cell-based biosensing, and allow for high-density microscopic analysis with simplified image processing.
Figure 2.10 Shear stress distribution in sidewall microgrooves for different width and pole radius \( W_c^* = 3.3, 2.8 \), and \( R_p^* = 0.625, 0.875, 1.125, \) and \( 1.375 \). The schematic of specified paths which shear stress distribution shown along the horizontal lines a-a, b-b, and c-c ([289] with permission)
Figure 2.11 Shear stress distribution in sidewall microgrooves for different width and pole radius $W_c^*=2.3, 1.8$, and $R_p^*=0.625, 0.875$, and $1.125$. The schematic of specified paths which shear stress distribution shown along the horizontal lines a-a, b-b, and c-c ([289] with permission)
Figure 2.12  Effect of variation of width in the cell penetration for three different geometry setup with $R_p^* = 0.625, 1.25, \text{ and } 1.875$ within the sidewall microgroove. $X$, and $Y$ are normalized coordinate system located at the top center of microgroove ([289] with permission)
CHAPTER 3

PIEZOELECTRIC POLYMER-COATED MICROCANTILEVERS FOR CHEMICAL SENSING PLATFORM

The change in resonance frequency of a micro/nanomechanical cantilever can be used as an ultrahigh sensitive method for detection of chemical analytes and biomolecules attached to the surface of cantilevers. In this chapter, a unique polymer-coated piezoelectrically driven microcantilever for chemical vapor detection is proposed. Both the experimental evidence and theoretical rational of analyte vapor detection are presented by our developed system. The detection of ethanol vapor (as the analyte vapor of choice) by Fluoropel polymer, and aromatic epoxy resin (D.E.N. 431) coated microcantilevers is first investigated. Furthermore, mechanical quality factor (Q) of polymer-coated Microcantilever-based sensors were evaluated. The details of mathematical modeling of piezoelectrically-driven micro/nanomechanical cantilever used in the experiments are also provided. It is envisioned that the proposed work can have a great impact on the development of many applications such as artificial nose and bio-nanomechanical sensors.

3.1 INTRODUCTION

There has been a growing effort and interest in the development of micro/nanomechanical cantilever based sensors in recent years [25-27]. In the past decade, Microcantilever-based sensors have emerged as an extremely high sensitive technology for the detection of chemical analytes and biomolecules [25]. The versatile application range of Microcantilever-based sensors in different
industries such as chemical, biotechnology, pharmaceutical, environmental, food and beverage, forensic, manufacturing, security and defense has contributed to their development immensely [25, 26, 28, 69-82]. In addition, the primary benefits of the Microcantilever-based systems are their lower cost, higher sensitivity, integration with Micro/Nano electromechanical systems (MEMS/NEMS), array configuration, simplicity, smaller size, label free detection of biomolecules, microenvironment exploring capability, higher surface-to-volume ratio, continuous quality and process control, higher functionality and faster response [25, 29]. It is also possible to use Microcantilever-based sensors with array configuration as an electronic nose or artificial nose to detect odors, gases, vapors and volatile organic compounds [28, 29].

Microcantilever-based sensors can be operated in static or dynamic mode. In static mode, the deflection of microcantilever will change by adsorption of analyte [28]. This deflection can be measured and correlated to the amount and type of adsorbed substance. In the dynamic mode the resonance frequency of microcantilever will reduce due to additional mass loading by adsorbed analyte [30]. The sensitivity of the Microcantilever-based sensors in this mode of operation can approach to a sub-picogram resolution [29, 30]. Microcantilever-based systems are capable of real-time, multiplexed detection of analytes and biomarkers in a very small volumes of samples [33]. Current microfabrication techniques have enabled the integration of electronic readout and sample introduction into a single unit thereby reducing cost, decreasing the device size, and the detection time can be significantly shortened [33].

An excellent way of enhancing and broadening the applicability of Microcantilever-based sensors is to coat them with a layer of sensitive polymer [83]. As a result, these newly devised sensors and devices can be used as an ultrasensitive nanomechanical sensors for the detection of chemical and biochemical agents in both gas-phase and liquid environments [28, 84]. The sensitive polymer
layer can be highly specific to an analyte or it can be partially specific to that analyte [28]. Therefore, this sensitive coating layer can detect a wide range of analyte concentrations in the environment [29]. It is shown that some of organic polymers such as poly(vinyl acetate) (PVA), poly(iso-butylene) (PIB), or poly(ethylene vinyl acetate) (PEVA) will expand or contract as they are exposed to analyte vapor molecules [85].

Important advances have been made to use piezoresistive Microcantilever-based sensors in environmental applications, where these microcantilever sensors detect based on static bending [89]. However, the piezoresistive effect of these microcantilevers consumes milliwatts of power to make measurements thus generating unwanted local heat [89]. A better way to address these challenges is to use smart materials. One of the smart materials that can serve as sensor and actuator with low power consumption is piezoelectric material [34, 86, 87].

In this study a novel polymer-coated piezoelectric (ZnO) microcantilever for the chemical vapor detection based on piezoelectric self-excitation and self-detection mechanism is proposed. First, the detection of Ethanol vapor by one percent fluoropolymer solutions in low boiling point fluorosolvent, and aromatic epoxy resin (D.E.N. 431) coated microcantilevers was investigated. Furthermore, mechanical quality factor (Q) of polymer-coated Microcantilever-based sensors in our experiments were evaluated. The details of mathematical modeling of piezoelectric microcantilevers used in these experiments was provided. It is envisioned that our work can have great impact on development of many applications such as artificial nose.
Figure 3.1 a) Schematic representation of polymer-coated piezoelectric microcantilever b) Piezoelectric microcantilever measurement platform c) Microscopic image of microcantilever d) Magnified image of the microcantilever (Lower right scale bar: 20 μm )

3.2 EXPERIMENTAL SETUP

Piezoelectric microcantilevers were obtained from Bruker (Vecco Active Probe®, Bruker Nano Inc., CA). These microcantilevers are made of a silicon (Si) substrate which supports a Zinc Oxide (ZnO) stack with a layer of titanium and gold. The ZnO layer is sandwiched between gold and titanium layer [35, 292]. The schematic and microscopic image of piezoelectric microcantilevers are shown in Figure 3.1. A platform for mounting of the microcantilevers and performing measurements was devised. Figure 3.1b presents the layout of the measurement platform.
For the present studies, the surface of microcantilevers were coated with Fluoropel PFC polymer. Fluoropel PFC [88] were obtained from Cytonix (Cytonix, Beltsville, MD). Fluoropel PFC polymer is a hydrophobic, transparent film providing repellency to water, paints, organic solvents, corrosion resistance, and prolonged use at temperatures up to 200 degrees C. Fluoropel PFC is an FDA approved polymer and can be used in biomedical devices and other bio-related applications [88]. This polymer has the surface tension energy of about 16 dynes/cm and dries out at the room temperature after 4 to 6 hour of exposure to the air [88]. The surface coating of microcantilevers with Fluoropel PFC polymer was performed by introducing the microcantilever into the solution. Subsequently, the microcantilevers were left to dry out at the room temperature after 5 hours of exposure to the air. Figure 3.1a presents the schematic of polymer-coated piezoelectric microcantilever. Figure 3.2d represents the microscopic image of Fluoropel PFC coated microcantilever.

In addition, aromatic epoxy resins (DEN 431) for coating of microcantilevers was used. Aromatic epoxy resins was obtained from Dow Chemical Company. Aromatic epoxy resins (DEN 431) are produced by reacting epichlorohydrin with a low molecular weight phenol-formaldehyde resin [90]. Epoxy resins (DEN 431) has multi-epoxy functionality. Therefore, this makes DEN 431 resins especially useful in adhesives, electrical and structural laminates, coatings and castings for elevated temperature service [90]. To get a variation in viscosity of aromatic epoxy resin, the polymer was blended with the acetone solvent. Therefore, one volume of the epoxy resin to four and three volumes of acetone was introduced, respectively. After applying the polymers to the surface of microcantilevers they were placed in oven for two hours at 300 °F.

Microcantilever mounted platform was placed in the air-tight glass cell for performing experiments. Detection of analyte vapors happens via diffusion of the vapor molecules into the
polymer coating of the microcantilevers. Our platform consists of four microcantilevers, one bare microcantilever, one PFC coated, and two aromatic epoxy resin coated microcantilever. One configuration of these microcantilevers are shown in Figure 3.1b. The images of each polymer coated microcantilever is presented in Figure 3.2.

In the present experiments, microcantilevers are vibrated at their fundamental resonance frequency. This excitation happens through the integrated piezoelectric film actuator. Furthermore, the fundamental resonance frequency of the polymer coated microcantilevers were measured by a laser vibrometer. A CLV-2534 compact laser vibrometer from Polytec to achieve this objective was employed. The fundamental resonance frequency of the microcantilevers was also measured by a bridge circuit system.

3.3 RESULTS AND DISCUSSIONS

This work presents both the experimental evidence and theoretical rational of analyte vapor detection by polymer coated piezoelectric microcantilevers. First, the change of fundamental resonance frequency of polymer coated microcantilevers due to adsorption of analyte to the polymer layer was investigated. To achieve this objective, a laser vibrometer to perform our measurements was employed. Moreover, to gain further insight into the shift of resonance frequency of polymer coated microcantilevers, additional measurements by a bridge circuit system was performed. In our impedance bridge circuit configuration the piezoelectric microcantilever is connected as one arm of the impedance bridge. The excitation of microcantilevers happens by applying harmonic voltage to the impedance bridge.
An Agilent oscilloscope with built-in function generator was used for excitation of the microcantilevers and sweeping the drive frequency through the cantilever resonance. The driving frequency for the laser vibrometer measurements were in the frequency range of 0-100 kHz. In case of impedance bridge circuit measurements, the driving frequency were in the frequency range of 0-12 MHz. Ethanol vapor was used as our analyte vapor of choice. Specifically, our investigations and measurements were done by a pre-exposure stage (measurements in air), exposure stage (measurements in Ethanol vapor), and post-exposure stage (measurements in air again). Here, experimental observations of frequency response of these polymer-coated piezoelectric microcantilevers when exposed to the air and Ethanol vapor is presented. The results of these measurements are shown in Figure 3.3A-D. This figure represents frequency response of the piezoelectric microcantilevers exposed to Air (Red line), Ethanol (black dashed line), and post-

Figure 3.2 Microscopic images of the microcantilever a) bare microcantilever b, c) microcantilever coated with aromatic epoxy resin D.E.N. 431 d) microcantilever coated with PFC, Scale bar: 20 μm
exposure (Air again, Blue line) and measured by laser vibrometer. Figure 3.3 corresponds to the resonance frequency change of microcantilevers coated with (3.3A) aromatic epoxy resin DEN 431, (3.3B) less viscous aromatic epoxy resin DEN 431, (3.3C) no polymer or bare microcantilever, and (3D) PFC, respectively. The obtained frequency responses clearly exhibit a frequency change due to adsorption of ethanol to the polymer layer, as shown in Figure 3.3A, B, and D. The resonance frequency has reduced noticeably in case of aromatic epoxy resin DEN 431 coated microcantilevers (Figure 3.3A, B) when exposed to the ethanol vapor. However, there is a slight change of resonance frequency for PFC coated microcantilever for the same exposure to the analyte vapor (Figure 3.3D). For bare microcantilever there is extremely small change in the resonance frequency of microcantilever before or after exposure (Figure 3.3C). Experimental observation revealed that smaller resonance frequency change in the less viscous aromatic epoxy resin DEN 431 coated microcantilever will be experienced, Figure 3.3B, compared to the other epoxy resin DEN 431 coated microcantilever, Figure 3.3A. This phenomena is rationalized by noting that the less viscous epoxy resin DEN 431 coated microcantilever (Figure 3.3B) is thinner than the other epoxy resin DEN 431 coated microcantilever (Figure 3.3A). As a result, it is expected more analyte vapor will be adsorbed by the thicker polymer and consequently larger resonance frequency change happens.
Figure 3.3 Frequency response of the piezoelectric microcantilevers exposed to Air (Red), Ethanol (black dashed line), and post-exposure (Air again, Blue), coated with A) DEN 431 Resin B) less viscous DEN 431 Resin C) no polymer (bare) and D) PFC, respectively, and measured by laser vibrometer.

Thus, the polymer thickness should not be overlooked in polymer coated microcantilever design and measurements when analyte detection is deemed important. In addition, the slight change of resonance frequency of the PFC coated microcantilever may be related to its repellency of organic solvent and also the small deposition of PFC polymer on the microcantilever surface, as illustrated in microscopic image of it (Figure 3.2D).
To further assert and validate our findings, these experiments were repeated overall three times. The experimental results are presented in Figures 3.4 and 3.5, respectively. Consistent with our previous discussion, upon adsorption of analyte by the polymer coating the resonance frequency of microcantilever changes. The patterns noted in Figure 3 (A–D) are also evident in Figure 3.4 (A–D) and Figure 3.5 (A–D). This implies the resonance frequency will change noticeably in case of aromatic epoxy resin DEN 431 coated microcantilevers when exposed to the ethanol vapor.
(Figures 3.4A-B, 3.5A-B). In contrast, there is a slight change of resonance frequency for PFC coated microcantilever for the same exposure to the analyte vapor (Figures 3.4D, 3.5D).

As explained earlier, to further confirm and validate our measurements performed by laser vibrometer, additional measurements by a bridge circuit configuration was performed. The measured changes of resonance frequencies of polymer coated microcantilevers by bridge circuit configuration are shown in Figure 3.6 A-D. Figure 3.6 represent the resonance frequency change of microcantilevers coated with (6A) aromatic epoxy resin DEN 431, (6B) less viscous aromatic

![Figure 3.5](image)

**Figure 3.5** Frequency response of the piezoelectric microcantilevers exposed to Air (Red), Ethanol (black dashed line), and post-exposure (Air again, Blue), coated with A) DEN 431 Resin B) less viscous DEN 431 Resin C) no polymer (bare) and D) PFC, respectively, and measured by laser vibrometer.
epoxy resin DEN 431, (6C) no polymer or bare microcantilever, and (6D) PFC, respectively. These results confirm the obtained frequency response from laser vibrometer measurements as presented before.

![Graphs showing frequency response](image)

**Figure 3.6** Frequency response of the piezoelectric microcantilevers exposed to Air (Red), Ethanol (black dashed line), and post-exposure (Air again, Blue), coated with A) DEN 431 Resin B) less viscous DEN 431 Resin C) no polymer (bare) and D) PFC, respectively, and measured by bridge circuit system.

It is noted that similar trend is observed in change of resonance frequency consistent with the laser vibrometer measurements. As stated previously, bridge circuit measurements were repeated three
times for each case corresponding to laser vibrometer measurement. The obtained data are presented in Figures 3.7 and 3.8. Indeed, the patterns observed in Figure (3.6A–D) are also similar in Figure (3.7A–D) and Figure (3.8A–D).

To further evaluate the change of resonance frequency of each polymer coated microcantilever for different experiments, the peak frequency from the corresponding resonance frequency plot was extracted.

**Figure 3.7** Frequency response of the piezoelectric microcantilevers exposed to Air (Red), Ethanol (black dashed line), and post-exposure (Air again, Blue), coated with A) DEN 431 Resin B) less viscous DEN 431 Resin C) no polymer (bare) and D) PFC, respectively, and measured by bridge circuit system.
These changes are calculated to precisely assess the amount of increase or decrease in resonance frequencies and presented through Figures 3.9 to 3.11. First, the frequency change of polymer coated microcantilevers that are related to the pre-exposure (Air) and then exposure to ethanol was considered. These results are shown in Figure 3.9 and gathered by laser vibrometer measurements.

**Figure 3.8** Frequency response of the piezoelectric microcantilevers exposed to Air (Red), Ethanol (black dashed line), and post-exposure (Air again, Blue), coated with A) DEN 431 Resin B) less viscous DEN 431 Resin C) no polymer (bare) and D) PFC, respectively, and measured by bridge circuit system.
Figure 3.9 Change in resonance frequency of microcantilevers coated with polymer D1) DEN 431 Resin D2) less viscous DEN 431 Resin, no polymer (bare) and PFC, related to the pre-exposure (Air) and exposure to ethanol by laser vibrometer.

Figure 3.10 Change in resonance frequency of microcantilevers coated with polymer D1) DEN 431 Resin D2) less viscous DEN 431 Resin, no polymer (bare) and PFC, related to the exposure to ethanol and post-exposure (air) measured by laser vibrometer.
As expected, larger frequency changes in the D1) DEN 431 Resin, D2) less viscous DEN 431 Resin, no polymer (bare) and PFC, related to the pre-exposure (Air) and exposure to ethanol by bridge circuit configuration was observed. Second, the frequency change of polymer coated microcantilevers that are related to the exposure to ethanol and post-exposure (air) and were measured by laser vibrometer was considered (Figure 3.10). It was noted that the changes in frequency follow the same pattern of Figure 3.9. Lastly, the changes in the resonance frequency observed in bridge circuit measurements was plotted (Figure 3.11). However, it was observed that frequency change in PFC coated microcantilever is relatively higher than the aromatic epoxy resin DEN 431 coated microcantilevers. This might be attributed to change of impedance of PFC coated microcantilever due to adsorption of ethanol to its surface. Furthermore, the quality factor of the polymer coated microcantilevers was evaluated. Microcantilever quality factor characterizes the shape of its frequency response curve near its resonance mode [91].

Figure 3.11 Change in resonance frequency of microcantilevers coated with polymer D1) DEN 431 Resin D2) less viscous DEN 431 Resin, no polymer (bare) and PFC, related to the pre-exposure (Air) and exposure to ethanol by bridge circuit configuration.
one can mathematically define the quality factor of i-th mode as the ratio of the resonance frequency of the i-th mode, \( f_i \), to the full width of the resonance peak evaluated at the half-maximum of the peak [91, 93-95]. Figure 3.12 illustrate the frequency response curve of aromatic epoxy resin DEN 431 coated microcantilever for ethanol detection, inset shows the mathematical definition of quality factor. Figure 3.13 presents the calculated quality factor of microcantilever coated with polymer D1) DEN 431 Resin D2) less viscous DEN 431 Resin and PFC. This graph reveals that the calculated quality factor in these cases are varying from 30 to 120 range. Since the geometry of our microcantilevers are the same, therefore these variation can be related to the damping effects [91]. Moreover, the quality factor in our range allows for a frequency resolution below 10 Hz [91].
To this end, experimental observations of analyte detection by the pizeoactive microcantilevers was presented. Here, a theoretical model to rationalize the experimental findings will be employed.

### 3.4 Modeling Piezoelectric Microcantilever Beam

A piezoelectric microcantilever beam can mathematically be modeled by considering the following assumptions. It is assumed that the Euler-Bernoulli theory can be applied to derive the governing equations [97, 98]. It is also assumed that the microcantilever beam has a non-uniform cross section, and linear system properties can be considered for modeling [100]. It is assumed that ZnO layer is attached to the top of the microcantilever beam as is shown in Figure 3.14. Furthermore, distributed-parameters system can be used for the modeling of microcantilever beam and attached ZnO layer [96, 97]. Moreover, equation of motion can be derived from extended Hamilton’s principle [97]. Therefore, the equations of motion can be written as follows [97]:

**Figure 3.13** Quality factor of microcantilever coated with polymer **D1**) DEN 431 Resin **D2**) less viscous DEN 431 Resin and PFC, respectively
\( \rho A \ddot{\eta}(x, \tau) + Q''(x, \tau) = 0 \) \hspace{1cm} (3.1)

where \( \rho \) is the combined volumetric density of beam and piezoelectric element, \( Q \) is the cross-sectional bending moment acting on the beam, \( \eta \) is the transverse beam deflection, \( \tau \) refers to time, and \( x \) refers to coordinate \([87, 92, 96-100, 101]\). A schematic representation of piezoelectric microcantilever with related coordinates is depicted in Figure 3.14. By substituting the relevant terms for \( Q \) in the equation (3.1), the following equation can be obtained \([96, 97]\):

\[
\rho A \ddot{\eta}(x, \tau) + \frac{\partial^2}{\partial x^2} \left( EI(x) \eta''(x, \tau) \right) = \frac{\partial^2}{\partial x^2} \left( -0.5 \frac{b E_p d_{31} U(t)(h_p + h_b)(h_p - 2 y_n)}{h_p(E_p h_p + E_b h_b)} \right) U(t) H''(x) \] \hspace{1cm} (3.2)

where \( E_p \) is piezoelectric elastic modulus, \( E_b \) is microcantilever elastic modulus, \( h_b \) is microcantilever beam thickness, \( h_p \) is ZnO layer thickness, \( E_b \) is microcantilever elastic modulus, \( d_{31} \) is piezoelectric constant, \( b \) is piezoelectric width, \( y_n \) is shift in the neutral axis, \( H(x) \) is the boxcar function, \( E \) is elastic modulus, \( I \) is moment of inertia, and \( U(t) \) is voltage applied across the actuator. In equation (3.2), overdot represents derivative with respect to time, and overprime
represents derivative with respect to spatial coordinate. In Right Hand Side (RHS) of equation (3.2), the term in the parenthesis is a concentrated moment at the boundary of the actuator. It is also possible to use the extended Hamilton’s principle and write the equations in the following form [96, 97]:

$$\rho A \ddot{\eta}(x, \tau) + \frac{\partial^2}{\partial x^2} (EI(x) \eta''(x, \tau)) + \gamma \dot{\eta}(x, \tau) + \varepsilon \eta'(x, \tau) = g(x, \tau) + Q_e''(x)$$  \hspace{1cm} (3.3)$$

where $\gamma$ is coefficient of viscous damping, $\varepsilon$ is coefficient of structural damping, $g(x, \tau)$ is a generalized external force, and $Q_e$ is the concentrated moment at the boundary due to actuator. With the boundary conditions:

$$\dot{\eta}(L, \tau) M_t - E_b I_b \eta'''(L, \tau) = 0$$
$$\eta(0, \tau) = \eta'(0, \tau) = \eta''(L, \tau) = 0$$  \hspace{1cm} (3.3a)\hspace{1cm} (3.3b)$$

where $L$ is the length of microcantilever, and $M_t$ is the tip mass. By considering the self-sensing effect [96, 97] in the piezoelectric microcantilever, and connected to a pure capacitance bridge the equation (3.3) can be rewritten in the following form [96, 97]:

$$\rho A \ddot{\eta}(x, \tau) + \frac{\partial^2}{\partial x^2} (EI(x) \eta''(x, \tau)) + \gamma \dot{\eta}(x, \tau) + \varepsilon \eta'(x, \tau) - \frac{Q_0}{Z_p + Z_1} J_s \left[ \eta'(x_2, \tau) - \eta'(x_1, \tau) \right] H''(x) = g(x, \tau) - U_0(\tau) \frac{Z_1 Q_0}{Z_p + Z_1} H''(x)$$  \hspace{1cm} (3.4)$$

where in equation (3.4), $U_0(\tau)$ is the voltage applied across the bridge and the $Z_1$ is the capacitance in the bridge, $Z_p$ is the effective capacitance of the piezoelectric element, $Q_0$ is a simplified term related to the concentrated moment at the boundary due to actuator. Furthermore, $x_1$ and $x_2$ refer to coordinate of beginning and ending of piezoelectric element, as depicted in Figure 3.14. $J_s$ is defined as follows:

$$J_s = \frac{1}{2} b E_p d_{31} (h_p + h_b)$$  \hspace{1cm} (3.5)$$

The equation of motion can be discretized by using Galerkin’s method.
\[ \eta(x, \tau) = \sum_{n=1}^{k} N_n(x) \xi_n(\tau) \]  

(3.6)

where \( N_n(x) \) are the eigenfunctions that satisfying the characteristic equation of the microcantilever and \( \xi_n(\tau) \) are the normalized coordinates and \( n \) is the number of modes. Therefore, the equation of motion can be represented in the discretized form as follows:

\[
\sum_{n=1}^{k} \left[ \int_{0}^{L} \rho(x) N_m(x) N_n(x) \, dx \right] \ddot{\xi}_j(\tau) + \sum_{n=1}^{k} \left[ \gamma \int_{0}^{L} N_m(x) N_n(x) \, dx + \varepsilon \int_{0}^{L} N_m(x) N_n'(x) \, dx \right] \dot{\xi}_n(\tau)
\]

\[
+ \sum_{n=1}^{k} \left[ EI(x) \int_{0}^{L} N_m''(x) N_n''(x) \, dx \right] \dot{\xi}_n(\tau)
\]

\[
- \sum_{n=1}^{k} \frac{Q_0}{Z_p + Z_1} f_s (N_m'(x_2) - N_m'(x_1))(N_n'(x_2) - N_n'(x_1)) \xi_n(\tau)
\]

\[
= \int_{0}^{L} N_m(x) g(x, t) \, dx - \frac{Z_1 Q_0}{Z_p + Z_1} U_0(\tau) (N_m'(x_2) - N_m'(x_1))
\]  

(3.7)

Equation (3.7) can be written in the matrix form as [96, 97]:

\[
\mathbf{\Lambda} \ddot{\mathbf{x}}(\tau) + \mathbf{\Psi} \dot{\mathbf{x}}(\tau) + \mathbf{\Omega} \mathbf{x}(\tau) = \mathbf{\Pi} U_0(\tau) + \mathbf{F} g_d(\tau)
\]  

(3.8)

where

\[
\mathbf{\Lambda}_{mn} = \int_{0}^{L} \rho(x) N_m(x) N_n(x) \, dx \quad m, n = 1, 2, \ldots k
\]

\[
\mathbf{\Psi}_{mn} = \beta \int_{0}^{L} N_m(x) N_n(x) \, dx + \varepsilon \int_{0}^{L} N_m(x) N_n'(x) \, dx
\]

\[
\mathbf{\Omega}_{mn} = \int_{0}^{L} EI(x) N_m''(x) N_n''(x) \, dx - \frac{Q_0}{Z_p + Z_1} f_s (N_m'(x_2) - N_m'(x_1))(N_n'(x_2) - N_n'(x_1))
\]

\[
\mathbf{\Pi} = -\frac{Z_1 Q_0}{Z_p + Z_1} (N_m'(x_2) - N_m'(x_1))^T
\]

By solving the discretized equation (3.8), following numerical results can be obtained.
3.5 NUMERICAL RESULTS

The obtained numerical simulations based on the mathematical formulation presented in the above section are presented here. The following physical and geometric parameters are considered for our modeling purposes: Microcantilever beam thickness, $h_b = 4 \, \mu m$, ZnO layer thickness, $h_p = 4 \, \mu m$, microcantilever elastic modulus, $E_b = 105 \times 10^9 \, Pa$, piezoelectric constant $d_{31} = 11 \times 10^{-12} \, C/N$, ZnO elastic modulus $E_p = 104 \times 10^9 \, Pa$, linear density of microcantilever beam 2330 Kg/m$^3$, length of microcantilever beam 470 $\mu m$, linear density of ZnO layer 6390 Kg/m$^3$.

![Figure 3.15 Frequency response of the piezoelectric microcantilever beam by numerical modeling](image)

The capacitance in the bridge $Z_1$, and the effective capacitance of the piezoelectric element $Z_p$ are considered to be $30 \times 10^{-12} \, F$. Figure 3.15 illustrate the obtained numerical result by solving discretized equations for piezoelectric microcantilever. The piezoelectric microcantilever’s first natural resonance frequency obtained from Figure 3.15 is 58.43 KHz. The obtained resonance frequency from the numerical modeling is in good agreement with our experimental observations measured by laser vibrometer. Furthermore, the first resonance frequency obtained from
experimental measurements and theoretical modeling was compared. The obtained data are presented in Table 3.1 and shows the consistency of the obtained values.

**Table 3.1** Comparison of the first resonance frequency of the piezoelectric microcantilever beam obtained from experimental measurements and theoretical modeling

<table>
<thead>
<tr>
<th>Approach</th>
<th>First Natural Frequency (KHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mathematical Modeling</td>
<td>58.43</td>
</tr>
<tr>
<td>Experiment</td>
<td>58.36</td>
</tr>
</tbody>
</table>

### 3.6 MEASUREMENT OF ADDED MASS

Nanogram detection of chemical analyte (ethanol) by measuring added mass to the surface of polymer-coated piezoelectric microcantilever beam was obtained. These values are related to the exposure of the microcantilevers coated with polymer D1) DEN 431 Resin D2) less viscous DEN 431 Resin, no polymer (Ref) and PFC to ethanol in three different experiments and are presented in Figure 3.16. To further validate our measurements, the amount of polymer (PFC) added to the surface of microcantilever by image analysis method and frequency shift method was calculated.
For image analysis method, grey scale images of polymer coated microcantilever and binarized images was used. Furthermore, a MATLAB code was devised to detect the edges of the PFC coated

![Image of microscope images and labeled images]

**Figure 3.17** A) Microscopic images of the microcantilever, B) grey scale image, C) edge detected image D) labeled image

**Table 3.2** Comparison of added PFC mass to the surface of the piezoelectric microcantilever beam obtained from frequency shift method and image analysis method

<table>
<thead>
<tr>
<th>Approach</th>
<th>Added Mass to the microcantilever (ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Image Analysis method</td>
<td>40.323</td>
</tr>
<tr>
<td>Frequency shift method</td>
<td>40.317</td>
</tr>
</tbody>
</table>
microcantilever images by using Sobel [102] edge-detection technique and labeling them. The obtained images are shown in Figure 3.17 (A-D). It was assumed that each of obtained circles in the edge-detected images are hemisphere polymers attached to the surface of microcantilever. Mass of each hemisphere can be obtained by calculating the volume of each hemisphere and multiplying it to the density of the polymer. The added mass through the frequency shift measurement method was also measured. The obtained values are in good agreement and presented in Table 3.2.

3.7 INTEGRATION OF PIEZOELECTRIC MICROCANTILEVERS WITH THE MICROFLUIDIC SYSTEM

An integrated microfluidic platform containing polymer coated piezoelectric microcantilever was devised and developed. This integrated device is composed of two parts, the first part is the microfluidic channel consisting of sidewall microchambers. The second part is a self-sensing piezoelectric microcantilever confined in the microchambers. This microfluidic system can be connected to the inlet and outlet ports for loading of specified reagents. The piezoelectric

![Figure 3.18 Schematic of the integrated microfluidic device containing microcantilevers](image_url)
microcantilever can be coupled to an excitation source (such as Agilent oscilloscope with built-in function generator) and bridge circuit system. Schematic of the integrated microfluidic device containing micro-cantilever is shown in Figure 3.18.

3.7.1 Microfluidic component

Both conventional soft lithography methods and three-dimensional (3D) printing methods for creating mold and channel of the microfluidic component were utilized. 3D printing is a form of additive manufacturing. This method usually utilized in manufacturing industries to produce design prototypes. Furthermore, this technology has been used in applications such as electronics, tissue engineering, and microfluidics [103]. A device is built by (3D) printing technology by laying down successive layer of material on each other. 3D printers use sliced three dimensional models that are created using a Computer Aided Design (CAD) software [103]. In this work, AutoCAD® software was used for initial design of the prototypes, and MeshLab open source software was used for viewing of generated STL files.

Figure 3.19 A) Computer design of device component in MeshLab, B) 3D printed device component
Computer design of device component in MeshLab, and 3D printed device component are presented in Figure 3.19. 3D printing technology offers a great deal of freedom for designing the device components and changing their features. Furthermore, this technology offers ease of fabrication and also provide quick production turn-around, allowing the design of such components to be altered, re-designed, and optimized rapidly [103]. 3D printed objects with different sizes, thickness, and features for both mold and channel of the fluidic component are shown in Figure 3.20. All of 3D printed objects were printed on 3D printers housed in the Department of Mechanical and Industrial Engineering at Northeastern University. Dimension 1200es 3D Printers from Stratasys™ (Eden Prairie, MN, USA) was used. Stratasys machines uses FDM (Fused Deposition Modeling) technology for 3D printing of objects. In these printers, 3D models are printed from the bottom up with precisely deposited layers of modeling and support material. Dimension 1200es 3D Printers use ABSplus™ modeling material. This material is a
production-grade thermoplastic and it is durable enough to perform virtually the same as production parts. Dimension 1200es 3D Printers are shown in Figure 3.21.

![Dimension 1200es 3D Printers from Stratasys™ (Eden Prairie, MN, USA)](image)

**Figure 3.21** Dimension 1200es 3D Printers from Stratasys™ (Eden Prairie, MN, USA)

### 3.7.2 Piezoelectric microcantilever component

Fabrication of Piezoelectric microcantilever components to the microfluidic system is a challenging task. It requires a technique for protection of small wires connected to the microcantilever system. In order to achieve this objective, a mold that fits the microcantilever setup by using 3D printing technology was devised. Then, the PDMS prepolymer mixed with silicone elastomer and curing agent (10:1) was poured on the mold and cured. PDMS conforms to the thin
wires very well. Next step, 3D printed molds were removed from the PDMS embedded microcantilever setup. Figure 3.22 represent piezoelectric microcantilever components ready for integration with microfluidic component.

**Figure 3.22** Piezoelectric microcantilever components ready for integration with microfluidic component

integration with microfluidic component.

### 3.7.3 Fully integrated system

The fully integrated system can be obtained by integration of piezoelectric microcantilever component with the microfluidic component. To achieve this goal, piezoelectric microcantilever component need to be carefully inserted in grooves of microfluidic component in order to reassure a good alignment. Inlet and outlet ports were connected to the tubing and sealed with quick drying...
epoxy. Figure 3.23 represent fully integrated microfluidic system by employing piezoelectric microcantilever.

More complex systems can be fabricated either by embedding smaller microcantilevers or array of microcantilevers in the grooves. Alternative physical set-up of the integrated microfluidic platform containing small microcantilever is presented in Figure 3.24. Furthermore, physical set-up of the integrated microfluidic platform containing microcantilever array is shown in Figure 3.25.
Figure 3.24 Physical set-up of integrated microfluidic system with embedded microcantilever component (left). Microscopic image of embedded microcantilever (right).

Figure 3.25 Physical set-up of the integrated microfluidic system with embedded microcantilever array (left). Microscopic image of microcantilever arrays (right)
3.8 CHAPTER SUMMARY

A unique polymer-coated piezoelectrically (ZnO) driven micro/nanomechanical cantilever for chemical vapor detection was experimentally demonstrated. Through a combined theoretical modeling the experimental findings were rationalized. Two different polymers, namely, aromatic epoxy resin DEN 431 and PFC were used for coating of microcantilevers. Ethanol was detected as the analyte of choice. The mechanical quality factor of polymer-coated microcantilever-based sensors in the experiments were evaluated and illustrated to be in the range of 30 to 120. Furthermore, the change of resonance frequency of each polymer coated micro/nanomechanical cantilever to precisely assess the amount of increase or decrease in resonance frequencies were investigated. Nanogram detection of chemical analyte (ethanol) by measuring added mass to the surface of polymer-coated piezoelectric microcantilever beam was obtained. Integrated microfluidic platform containing polymer coated piezoelectric microcantilever was presented. Furthermore, the amount of polymer (PFC) added to the surface of microcantilever by image analysis method and frequency shift method was determined. Hence, it is possible to use the simple and adaptable proposed platform as an electronic nose or artificial nose to detect odors, gases, vapors and other analytes. These demonstrations also suggest that the polymer-coated piezoelectrically driven micro/nanomechanical cantilevers are ideally suited for a sensitive detection of a wide range of analytes.
CHAPTER 4

AUTOMATED CELL SEGMENTATION METHOD FOR CELL COUNTING IN MICROGROOVE BASED MICROFLUIDIC DEVICE

In this chapter, a cell segmentation technique will be discussed for cell counting and extracting the cell information in a microgroove based microfluidic device. First, the microfluidic setup and phase contrast microscopy are introduced. Furthermore, the proposed image segmentation method is described and the process of cell analysis and counting are introduced. Finally, the results are presented.

4.1 INTRODUCTION

Technological advancement in cell and microscopy imaging generate a high demand for complex image analysis techniques. Image processing techniques are powerful tools to assess the images and extract information for quantization and evaluation of regions. It is crucial to develop advanced automatic image processing methods with capability to analyze image data with high level of accuracy. The field of image processing covers a broad range of operations to generate desired images or extract higher level information for vital applications. One of the application of image processing is cell counting. Manual cell counting is a time consuming and tedious task. Automated cell counting techniques reduces human errors and expedite the counting process. In this chapter a quantitative technique is developed to count the number of cells. The technique selects region of interests (ROI) in the cell images and it measures the cell locations within ROI in a specific microfluidic device.
4.2 MICROFLUIDIC SETUP AND PHASE CONTRAST MICROSCOPY

A PDMS-based microfluidic device was developed for cell based applications. The schematic of the device is shown in Figure 4.1 for the cell experiments. This microfluidic device mainly consisted of grooves and posts that enabled the control of flow velocity and shear stress profiles. A fluidic channel containing grooves and a PDMS substrate were irreversibly bonded by using oxygen plasma. Cells were seeded into a microfluidic device through a cell inlet port and medium was subsequently infused by using a syringe pump.

Cell images (phase contrast images) were obtained by using an inverted microscope (Nikon, USA). Phase contrast microscopy is a contrast-enhancing optical technique that can be utilized to produce high-contrast images of transparent specimens, such as living cells (usually in culture), microorganisms, thin tissue slices, lithographic patterns, fibers, latex dispersions, glass fragments, glass fragments,

![Figure 4.1](image)

**Figure 4.1** Microfluidic channel with microgrooves. (A) Schematic of the microfluidic channel containing the grooves; (B) Phase contrast image of grooves in a microchannel (top view, groove width 200 µm) and illustration of geometric dimensions of the device: Wc, half of channel width, Rp, post radius, Lg, groove length, Wg, groove width. ([289] with permission)
and subcellular particles (including nuclei and other organelles). In Figure 4.2 a sample of living cells in bright field and phase contrast are presented [104].

**Figure 4.2** (Left) Living cells in brightfield (Right) Phase contrast [104]

**Figure 4.3** A representative image taken from grooves located in the bottom row. The groove is divided into several sub-grooves and associated binary images are generated for cell counting ([290] with permission)

**Figure 4.4** A representative image taken from grooves located in the top row. The groove is divided into several sub-grooves and associated binary images are generated for cell counting ([290] with permission)
In order to predict the flow pattern and cell behavior in microfluidic channel, it is necessary to model the velocity field and stream line distributions. This can be achieved by using numerical simulation to solve the laminar flow governing equations for desired geometry [58, 59]. These have been discussed in detail in Chapter 2. It was shown that the cell location can be correlated to the shear stress distribution in the microfluidic device. Hence, an algorithm for counting the number of cells, measuring their locations and areas is required.

4.3 IMAGE SEGMENTATION

Segmentation subdivides an image into its constituent regions or objects. The level to which the subdivision is carried depends on the problem being solved. That is, segmentation should stop when the objects of interests in an application have been isolated.

Image segmentation is typically used to locate objects and boundaries (lines, curves, etc.) in images. More precisely, image segmentation is the process of assigning a label to every pixel in an image such that pixels with the same label share certain visual characteristics. It must be mentioned that image illumination, blurring and artifacts affect the segmentation results.

There are several techniques for image segmentation such as, clustering and classification methods, histogram shape-based techniques, entropy-based methods, and attribute-similarity techniques. Spatial and feature-based methods, local and global techniques, hybrid methods, and intelligent methods are part of segmentation techniques.

Clustering methods generate separate regions in the image such that a pixel in a given region does not belong to other regions. Classifications techniques generate classes in such a way that objects in same class have high degree of similarity. Objects belong to different classes have lower degree
of similarity. Usually, the classification techniques require training data with known labels. Histogram based techniques use some global characteristics of the image, such as brightness distribution. Some of the entropy-based methods employs two probability distribution related to the object and background. These probability distributions are derived from the original gray-level distribution of the image [105, 106].

The similarity between the binary image and original image can be used in attribute-similarity based methods and can lead to better object detection. The edges, shapes, connectivity, or compactness could be the attribute of interest. Features and spatial characteristics of the image can be applied in spatial and feature-based methods. In the spatial-based techniques the points of an object are considered spatially close which helps to segment the image in many cases. Local techniques are based on operation on the local parts of the image. Global methods are based on information in the whole image. The features of the objects in the image are a useful source of information in global techniques. Hybrid techniques are based on a combination of local and global characteristics of the image. Intelligent methods use artificial intelligence and machine learning techniques such as artificial neural networks [105, 106].

These segmentation methods [113, 114] have been largely implemented for cell counting and analysis. Some of the cell segmentation algorithms are based on image intensities and some others are region-based segmentation. Edge-detection techniques like Sobel, Marr [102], Canny [115] methods are widely used for cell segmentation. Chen et. al. presents edge-detection technique using illumination and contrast invariant measurement of edge significance [107].

Angulo et. al. present morphological [117, 119] based adaptive segmentation technique using watershed [116, 118, 120] transformation for fluorescence-labeled cell assays images [108]. The
segmentation of individual cells is based on the watershed technique using inner marker for each object of interest and a global outer marker for the background. In watershed techniques, grey level of a pixel is considered as an altitude. An image is considered as topographic relief which presents the amount of topographic change within the image. Hence, in watershed algorithm it is assumed that water flows in a path to reach a local minimum. Typical watershed algorithm are based on local minima of the gradient of the image or based on the marker positions defined by the user or morphological operators [108].

Bak et al. presented a segmentation method based on local information of the images. A criterion function is defined using statistical structure of the objects in cell image. Each pixel is assigned to the most probable region, and the assignment process is iteratively updated by criterion function until steady state is reached. Global and local characteristics of each object like nucleus and background of the cell are used for segmentation. Local spatial likelihood is defined for presenting global and local characteristics of the image [109].

Cheng et al. presented inner and outer cell contours edge used in parallel. A detection fusion algorithm combines the two detection results and increases the probability of cell detection. The algorithm is based on multiple cell detection and fusion. Several steps like pre-processing for masking of areas outside the ROI, edge detection, and cell segmentation using distance transform and watershed transform are implemented [110].

Vromen et al. presented a model-based contour tracing method for segmenting images of red blood cells. They used guided sequential trace of cell contours by using brightness changes in the images. They also incorporated knowledge about the contour shapes [111].
The selection of cell segmentation techniques is based on the application in hand. The first task of the proposed algorithm is the selection of region of interest by user input and extraction of features for image segmentation. Hence, at the beginning of the algorithm user selects region of interest which is a groove that is located at the top or the bottom of the image [290, 293, 294].

User selects the bottom groove or top groove by two clicks at top and bottom of the selected regions (Figurers 4.3 and 4.4). Then user selects the sub-grooves by clicking to the left and right of each sub-groove in obtained image. The sub-images (sub-grooves) are extracted and used for segmentation. Since the size of the grooves and number of sub-grooves are different in the images, the user input can generate an adaptive technique which works for all groove sizes. The algorithm creates a corresponding black image with the same size of sub-groove.

Feature selection is an important step for image segmentation. User clicks on the center of one cell in each sub-groove to extract the location and intensity of the center of the cell. In each sub-groove, the algorithm calculates the size of the sub-groove and saves the value.

Algorithm, automatically finds all the pixels that has the same intensity of the clicked area and check whether their neighborhood pixels (right and right down) have similar intensities. Moreover, the algorithm changes the associated pixel values in the associated black image to the white pixels and fills the holes in the generated binary images. Finally, it saves the cell centers and areas in a vector. The proposed method uses the binary image to count the number of the cells. The details of the algorithm are presented in the Table 4.1. Figures 4.5 and 4.6 demonstrate the steps of the algorithm with generated results.

As mentioned above the image intensity and location extracted from the clicked area are used as segmentation parameters which generate the optimal results. The algorithm was developed by using
MATLAB programming language. The MATLAB command “bwlabel” was applied after binarization step for each sub-groove with binary image as input to label the binary image and count the number of the cells. Moreover, MATLAB regionprops calculates area and centroid for each cell using labeled binary image.

Bwlabel, Labels connected components in binary image and regionprops measures a set of properties for each labeled region. The following MATLAB codes present the process of extracting sub-groove and cell information from binarized images.

```matlab
MixImg = imfill(MixImg,'holes'); //Filling the holes in binarized sub-groove//
[LPram, NumberCells] = bwlabel(MixImg,8); //labeling binarized sub-groove//
Grooves{numgg,4} = regionprops(LPram, 'Area', 'Perimeter','Centroid','BoundingBox'); //Calculating area and centroid of the cell from binarized sub-groove//
Grooves{numgg,5} = size(MixImg); //Calculating the size of binarized sub-groove//
```

To further validate the method, the number of cells generated by the proposed technique is compared to the number of cells counted manually and number of cells obtained by ImageJ. ImageJ is public program based on Java for image processing and analysis and it is developed by National Institutes of Health (NIH). ImageJ has capability to perform image processing and computer vision tasks such as reading images with different formats. It can display, edit, analyze, process, and save images [112].

4.4 RESULTS

The segmentation algorithm was applied on 97 images extracted as regions of interest. In order to validate the result (as it is presented in Table 4.2), the number of cells calculated from the proposed technique is compared with the number of cells that manually counted. In 83.51% of the cases the number of cells from the image is equal to the number of cells counted manually. In 13.4% of
cases the difference between number of cells from the proposed technique and number of cells counted manually was one cell. The reason of this difference is partial location of the cells in the extracted sub-groove borders or the small size of the cells with very low intensity which more likely related to cells that are not alive.

In 3.1% of the times the difference of manually counted cells and number of cells from method is more than 1 cell. One other reason for the underestimated number of cells is the generation of the clumps of the cells. In some binary images, the clumps appear as one cell.

Figure 4.5-(I) presents sample of original image with associated binary image generated by the proposed technique.

Figure 4.5-(II) also presents sample of the results generated by the proposed method with underestimated results. The arrows shows the cases that a cell is very small and not detected in the binary image or several cells generated a clump appeared as one cell in the binary result. Figure 4.5-(III) part C shows a sample overlaid images generated by original image (part A) and its associated binary image (part B). The reason for overlaying those images is that to verify the position of cells in the binary image is the same as the original image.

Figure 4.5-( IV) represents a comparison of the binary images generated from proposed method (part B) with the results of binary image generated from ImageJ using make binary functionality (part C), and Image-adjust-threshold functionality (part D). Make binary option in ImageJ does not produce a satisfactory result for cell counting. Figure 4.5-(IV) part D images were generated by manually changing two sliders in the ImageJ software until a satisfactory binary image was generated for each case. However, this process is very time consuming. Table 4.3 presents number of the cells obtained by the proposed technique, ImageJ, and manual counting.
Moreover, the size of the sub-grooves, the area and centroid of each cell that calculated from the method are shown in Table 4.3.

Table 4.1 Segmentation algorithm for cell counting

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<td>Read Image from file</td>
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<tr>
<td>Select region of interest from up or down groove by click</td>
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<tr>
<td>Select sub-grooves from up or down grooves</td>
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<tr>
<td>For each sub-groove</td>
<td>Create corresponding black images with the same size of sub-groove</td>
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<tr>
<td>In each sub-groove</td>
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<tr>
<td>- Calculate the size of the sub-groove and save the value</td>
<td>- Click on the center of one cell</td>
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<td>- Extract the pixel intensity and coordinate of clicked area</td>
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<td>- Find the pixels that has the same intensity of the clicked area and check whether their neighborhood pixels (right and right down) have similar intensities</td>
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<td>- Change the associated pixel values in the associated black image to the white pixels</td>
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<td>- Fill the holes in the generated binary images</td>
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<td>- Save the cell centers and areas in a vector</td>
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<td>Count the cells in all binary images</td>
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Table 4.2 Results obtained from proposed method (automated cell counting) vs. data obtained from manual counting; Im1 denote the index of image; for grooves, G1 to G8 denote the subimage of the desired grooved. md denote data obtained from applying method (automatic counting) and cn denote data obtained from manual counting. up and dn denote top and bottom grooves, resp. dashed entries means there is no value.

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<th>G3 # cells</th>
<th>G4 # cells</th>
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Figure 4.5 (I) A. Original image; B. Binary images generated by the proposed technique. (II) A. Original image; B. Results generated by the proposed method which present the underestimated cases. The arrows present the cases that a cell is very small and not detected in the binary image or several cells generated a clump appeared as one cell in the binary image. (III) A. Original image; B. Binary image generated by the proposed technique.; C. Overlaid images generated by original image (part A) and its associated binary image (part B). (IV) A. Original image; B. Binary images generated by the proposed technique; C. Comparison of the binary images generated from proposed method (Part B) with the results of binary image generated from ImageJ using make binary functionality in the software; D. Comparison of the binary images generated from proposed method (Part B) with the results of binary image generated from ImageJ using Image-adjust-threshold ([290] with permission)
Table 4.3 Number of cells obtained from proposed technique, imagej, and manual counting. The size of the sub-grooves, the area and centroid of each cell calculated from the method

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4.5 CHAPTER SUMMARY

In this chapter, a cell segmentation technique was developed for cell counting and extracting the cell information in the proposed microfluidic device. The technique provides a tool for the selection of the region of interests (ROI) in the cell images and measurement of the cell locations within ROI. Furthermore, the number of cells calculated from the proposed technique is compared with the number of cells that manually counted for the total of 97 (images) sub-grooves extracted from the original images. In 83.51% of the cases the number of cells from the image is equal to the number of cells counted manually. In 13.4% of cases the difference between number of cells from the proposed technique and number of cells counted manually was one cell. The reason of this difference is partial location of the cells in the extracted sub-groove borders or the small size of the cells with very low intensity which more likely related to cells that are not alive. In 3.1% of the times the difference of manually counted cells and number of cells from method is more than 1 cell. This can be due to the attachment of the cells to each other. All of the coordinates of cell location has been extracted. Overall, this work provides a tool for extracting information from obtained images for further fluidic analysis.
CHAPTER 5

NANOPARTICLE GENERATION WITH FLOW FOCUSING MICROFLUIDIC DEVICE

In this chapter, first an overview of different methods for synthesis of nanoparticles will be described. The concept of flow focusing microfluidic device will be explained. Furthermore, capability of nanoparticle synthesis by our microfluidic platform have been demonstrated. Finally, the exploratory portion of nanoparticle generation demonstrating the synthesis of two different nanoparticles, namely, gold and liposomes by using flow focusing microfluidic technique was discussed.

5.1 INTRODUCTION

Microfluidic devices hold great promise for nanoparticle synthesis and high-throughput screening [12, 22, 23, 38, 40, 121-123]. They enable the controlled manipulation of small amounts of fluid, important when costly reagents are employed [11, 39, 40, 46, 123-125]. Microfluidic devices are also able to rapidly mix reagents, provide homogenous reaction environments, control reaction temperature, and allow addition of reagents at precise time intervals. These features make the system suitable for nanoparticle synthesis [24, 126]. Moreover, the simplicity and reproducibility of microfluidic device fabrication and handling of small volumes with less expensive materials makes it an ideal platform for rapid synthesis and optimization of nanoparticles. In addition, by recent development in the field it is possible to carry out in-line characterization, feedback control, and continuous synthesis of nanoparticles that will contribute to the optimization and screening of libraries of nanoparticles [129-131]. There are some evidence that by using microfluidic devices
for nanoparticle synthesis a narrower size distributions, and improved batch-to-batch reproducibility can be achieved [127, 128].

5.2 NANOPARTICLE SYNTHESIS METHODS

There are many different ways that nanoparticles (NP) can be synthesized. This depends on the type of the materials that will be used for synthesis or the desired shape of the nanoparticle. One possible way to categorize the NP synthesis is based on the fact that it is done in a bulk/ bench-top manner or microreactor manner. Classification of NP generation and synthesis methods is provided in Figure 5.1.

5.2.1 Bulk synthesis methods

Bulk synthesis of organic NP’s can be divide into two main approaches [135, 136, 137], two-step procedures and one-step procedures [133]. The two-step procedures involves the preparation of an emulsification system for generating nanodroplets, and then followed by the second step of forming NP’s through different mechanisms including gelation, polymerization and precipitation [133]. In one-step procedures there is no need for emulsification prior to NP formation. Examples of one-step procedures are self-assembly methods, nanoprecipitation by solvent displacement, and ionic gelation methods [133].

Two-step procedures:

Two-step procedures are mostly based on emulsification. Broadly speaking, emulsion can be considered as mixing of two or more immiscible liquids or dispersing of one fluid into another nonimmiscible one with the presence or absence of a surface active agent [133, 208]. The emulsification methods itself can be divided as low energy or high energy emulsification methods [133]. In low energy emulsification methods, nanoemulsions can be generated by spontaneous
emulsification which originate from the interfacial turbulence related to surface tension gradient (Marangoni effect) between two fluids [133, 138-143]. On the other hand, high energy emulsification methods involves nanoemulsions that can be generated by high-energy stirring methods or sonication methods [133, 144-145]. Schematic of emulsification by stirring is shown in Figure 5.2.

Several methods such as solvent evaporation, solvent diffusion, or salting-out procedures have been developed to perform the two-step NP generation procedures.

![Figure 5.1 Classification of NP generation and synthesis methods](image-url)
Solvent Evaporation:

In this method, a polymer will be dissolved in an organic or volatile solvent first and the obtained phases will be emulsified [98]. The next step would be the removal of the organic solvent through evaporation. The organic solvent can be evaporated either under the vacuum or by increasing the temperature. Most widely used solvents for this procedure are chloroform, Dichloromethane, and ethyl acetate [133, 146, 209].

Solvent Diffusion:

The solvent diffusion method also called solvent displacement method or nanoprecipitation [133]. In the solvent diffusion method a polymer will be dissolved in a partially water-soluble solvent. Subsequently, the obtained phases will be emulsified rapidly and leading to the solvent diffusion and nanoparticles formation [147, 148, 209].

Salting-Out:

Salting-out method is a variation of solvent diffusion technique [210]. In principal, adding a water-miscible solvent to an aqueous phase containing electrolytes will result in the separation of water-miscible solvent [210]. This phenomena is called salting-out effect. The preparation of NP’s can
be achieved by separation of water-miscible solvent from aqueous solutions by salting out effect [133, 149-151, 209, 211].

**Gelation:**

It is possible to obtain nanoparticles after nanoemulsification by gelifying of polymer or crystalize lipid dissolved in the droplets. For instance, gelatin and agarose NP’s can be produced by raising the temperature above their melting point and subsequently decreasing the temperature to induce the gelation and finally leading to NP generation [133, 213, 214]. Ionic gelation can also be performed for the synthesis of NP’s [212]. Solid lipid nanoparticles have also been produced by crystallization of the lipid under the melting temperature [133, 215].

**Polymerization:**

Polymerization from emulsion is a well-established method for NP generation with desired properties. Generally, polymerization for NP generation involves two stages. First stage is the micronization of material into NPs, and then followed by the second stage of stabilizing the generated NPs [212]. Micronization can be started with methods such as monomer polymerization where macromolecules form through polymerization of monomers. Emulsion polymerization method can be classified into methods such as conventional emulsion polymerization, surfactant-free emulsion polymerization, mini/micro-emulsions polymerizations, interfacial polymerization, and controlled/living radical polymerization [133, 212]. Various type of NP’s has been produced by conventional emulsion polymerization [216-218]. In surfactant-free emulsion polymerization method emulsifier is no longer needed. Therefore, the surfactant removal step is removed from the process [153, 219, 220]. In mini/micro-emulsion methods, direct polymerization of monomer droplets is the main advantage of the method over the conventional emulsion polymerization method [221]. This method is extremely useful for the polymerization of water insoluble
monomers [154, 221-225]. In interfacial polymerization, the polymerization happens at the interface of two immiscible fluids [226]. This technique is a well-known method for NP generation [227, 228]. Controlled/Living Radical Polymerization (CLRP) methods [229] can also be employed to generate NPs and precisely control the macromolecular architecture and end-functionalities of them [155, 230, 231].

**One-Step Procedures:**
One-step procedures can be defined as procedures that there is no need for emulsification prior to NP formation. Several methods such as nanoprecipitation, dialysis, desolvation, self-assembly methods, and ionic gelation methods are developed to perform one-step procedures [133].

**Nanoprecipitation:**
This method is similar to the previously described solvent diffusion method. However, in this method the emulsification and polymer precipitation are spontaneous. Therefore, NP’s can be formed spontaneously without external energy input in this process [232, 142].

**Dialysis:**
Dialysis technique is a simple and effective method for NP generation [156, 233]. In this method, the dissolved polymer will be placed in the dialysis membrane with some certain molecular weight cut-off [135]. The dialysis membrane provides a physical barrier for the polymer in this technique. Dialysis method can be used for preparation of NP’s which are made of materials such as graft copolymers, cellulose and block-copolymers [156, 234-235]. In dialysis technique, morphology and particle size distribution of the nanoparticles will be affected by the solvent used in the preparation of the polymer solution [135, 236].

**Desolvation:**
This method is based on a thermodynamically driven self-assembly process for nanoparticle preparation particularly for polymeric materials [212, 237-238]. This method is generally
employed for the production of nanoparticles of different types of proteins such as bovine serum albumin (BSA), human serum albumin, and gliadin [157-158, 212, 239]. In this method, different parameters such as the amount of desolvation agent, the ionic strength, protein content, and the agitation speed play a key role in the formation of nanoparticles with different sizes [212]. In addition, cross-linking may be applied simultaneously or subsequently to the desolvation step for stabilization of the generated nanoparticles [212].

Self-Assembly and Gelation:
A subgroup of one-step procedures can be classified as self-assembly and gelation techniques. This group of procedures can be divided into three mechanism of formation including: Ionic Gelation, Polyelectrolytes Complexation, and ‘‘Lock and Key’’ Nanogels.

Ionic Gelation:
In this approach, the nanoparticle generation can be performed in aqueous medium in very dilute solution and usually without surfactant. The nanoparticle are formed from cross-linking of the polymers due to electrostatic interaction. This cross-linking can be related to the interaction between two oppositely charged polymers or between counterions and charged polymers [137, 212]. Nanoparticles can be generated from two polymers such as ionic chitosan and sodium tripolyphosphate (TPP) by ionic gelation method [242]. By performing this technique, it is also possible to form chitosan nanoparticles coated with diblock PEO-PPO copolymer [243].

Polyelectrolytes Complexation:
The polymers that contain a net negative or positive charge at near neutral pH are called polyelectrolytes [240]. They are generally soluble in water and their solubility is driven by the electrostatic interactions between water and the charged monomer [240]. Polyelectrolytes Complexation (PEC) resemble ionic crosslinking since non-permanent networks are formed that
are more sensitive to environmental changes [244-245]. However, polyelectrolytes complexation interact between the polyelectrolyte and larger molecules with broad molecular weight range, this is in contrary to ionic crosslinking that ionic molecules react with polyelectrolyte [244-245]. The nanoparticles generated based on this method are widely used as in vivo drug delivery carrier of nucleic acids [159, 246].

*Lock and Key Nanogels:*

An attractive class of nanoparticles are so-called supramolecular nanoassemblies (nanogels). Nanogels are colloidal nanoparticles composed of cross-linked hydrophilic polymer network [247]. In general, one of the ways that colloidal systems can be formed is through the association of amphiphilic polymers in water, from the complexation of oppositely charged polyions or from hydrogen-bonding interactions [248]. Nanospheres can be formed by supramolecular nanoassemblies based on a “lock and key” concept. These polymers are composed of dextran modified by the grafting of alkyl chains and a poly(beta-cyclodextrin) [160].

*Nanoparticles Prepared by Drying Processes:*

The need for developing environmentally safer methods that does not require involvement of organic solvent for nanoparticle generation has resulted in advancement of drying processes [137]. These processes can be divided into supercritical and spray drying methods. Two techniques have been developed for the production of nanoparticles using supercritical fluids: (a) Rapid expansion of supercritical solution and (b) Rapid expansion of supercritical solution into liquid solvent [233].

*Spray-Drying:*

Spray drying is a transformation of feed from a fluid state into a dried particulate form by spraying the feed into a hot drying medium [251]. The main aim of drying by this method in pharmaceutical
technology is to obtain dry particles with desired properties [251]. This process consists of three steps: (a) atomization, (b) dehydration, and (c) powder collection. Practically, the liquid feed is atomized by an atomizer creating a spray of fine droplets into a chamber of heated air, from which the solvent quickly evaporates resulting in dried particles [249-250]. Nanoparticles can be generated by this technique from different polymeric materials including: polyvinyl alcohol (PVA), modified starch, Arabic gum, whey protein, and maltodextrin [252].

**Rapid Expansion of Supercritical Solution:**

In this method, the solute will be dissolved in a supercritical fluid to form a solution, then solution subsequently undergoes a rapid expansion through an orifice or a capillary nozzle directly into ambient air [135, 161]. The high degree of super saturation, accompanied by the rapid pressure reduction in the expansion, results in homogenous nucleation and thereby, the formation of well-dispersed particles [161]. In majority of studies, carbon dioxide is used as the supercritical fluid. Generally, a typical experimental device is composed of three components: a high pressure mixing chamber, a syringe pump, and a pre-expansion module [161, 254]. Poly-L-lactic acid (PLLA) and Poly(heptadecafluorodecyl acrylate) nanoparticles has been generated by this method [253, 255].

**Rapid Expansion of Supercritical Solution into Liquid Solvent:**

An elegant modification to the conventional Rapid Expansion of Supercritical Solution (RESS) process has been done by expanding the supercritical solution into a liquid rather than a gas [256-258]. This modification is called Rapid Expansion of Supercritical Solution into Liquid (RESOLV). This modification is done to address agglomeration concerns related to the conventional RESS method [256]. Furthermore, the liquid solvent apparently suppresses the particle growth in the expansion jet, thus making it possible to obtain primarily nanosized particles.
Poly(heptadecafluorodecylacrylate) (PHDFDA) particles were generated using water as the solvent in which were expanded the supercritical solution and precipitated the polymer [257]. Furthermore, Poly(methyl methacrylate) and poly(L-lactic acid) nanomaterials were also synthesized by this method using a CO2-cosolvent as the supercritical fluid [133, 258].

### 5.2.2 Microfluidic synthesis methods

Microfluidics and Microreactor based systems have enabled the synthesis of nanoparticles with narrower size distributions, and improved batch-to-batch reproducibility [22, 23]. Batch (Bulk) synthesis of nanoparticles tends to suffer from irreproducibility of size, size distribution, and quality of the nanoparticles from batch to batch [24]. It is also difficult to implement fast screening and optimization of the synthesis conditions in batch, and there are challenges in scaling batch procedures up to quantities needed for device development and optimization [24].

Some key advantages of microfluidics include simplicity and reproducibility of device fabrication and potentially lower cost of materials due to the ability to handle small volumes [22]. These advantages make the use of microfluidics ideal for the development of a platform that enables rapid synthesis and optimization of nanoparticles [22, 23]. The main challenge in applying microfluidics to materials synthesis is to design microsystems capable of producing the desired solid materials at the desired conditions (pressure, temperature, chemical compatibility, concentration) without the products clog the system [24]. The evolution of microreactors have been from simple tubing to more complex systems integrating control of operating parameters and in situ characterization techniques [24, 162-164].
Organic materials processing and synthesis in microsystems:

Polymerization reactions: Among the successful organic solvent-free routes to polymer synthesis, emulsion radical polymerization processes and photopolymerization hold significant importance [259]. Photopolymerization has indeed distinct advantages such as ambient temperature reaction, fast reaction rates, spatial and temporal control, and the possibility to use energy-saving lamps [259]. It is possible to combine microreaction technology and photopolymerization to generate polymeric nanoparticles [259]. Monodisperse microsized particles with controlled and complex morphology could be obtained by this approach as well [24, 165, 166, 259]. Anionic and cationic polymerization can also be performed in a microfluidic system [262, 263].

Precipitation of polymers:
Microfluidic techniques can be employed for synthesis of polymer particles based on precipitation created via solvent removal (for example by evaporation) or anti-solvent addition. Polymersomes and gel shells can be obtained through these techniques from double emulsions [24, 260, 261]. Nanoprecipitation by hydrodynamic flow focusing in a microfluidic system can also be employed for generating polymeric nanoparticles. Size-tunable PLGA-PEG nanoparticles can be synthesized by hydrodynamic flow focusing in a controlled nanoprecipitation process [22, 23]. Furthermore, conventional methods of preparing targeted nanoparticles involve a series of chemical processes whereby the nanoparticle core is initially formed, followed by the bioconjugation of targeting ligands to the surface of the nanoparticles. It is possible to use the micro reactor systems to alleviate the problems associate with the batch processing and generate targeted polymeric nanoparticles [126].
Crystallization of organic materials and proteins:
Crystallization of organic materials including small active pharmaceutical ingredients and proteins have benefited immensely from microfluidic systems [24, 167, 168]. Their advantage is the generation of droplets, every droplet being an independent crystallizer with volumes in the nanoliter range [264]. Growing of high-quality crystals of proteins and other macromolecules plays an important role in structural biology [265]. Several crystallization conditions and proteins can therefore be screened with nanoliters of volumes in microreactor system [169, 265]. Furthermore, these microfluidic crystallization tools make it possible to study nucleation in both aqueous and organic solvents, rendering microfluidic devices applicable to organic molecules such as active pharmaceutical ingredients (API) [264].

Inorganic micro and nanostructures synthesis in microsystems:
Microsystems have provided great opportunities for synthesizing inorganic nanoparticles in a continuous manner, with uniform and tunable size distribution, and controlled shapes. Microfluidic synthesis methods has been reported to be superior to batch synthesis methods for producing high quality, monodisperse particles due to the ability to maintain fine control of all solution variables including reactant concentration, timing of reagent addition, and temperature [266].

Synthesis of metal nanoparticles:
One approach that is employed for the synthesis of metal nanoparticles is through a microreactor at room temperature conditions and by mixing of an aqueous stream containing the metal salt with the reducing agent [24]. Therefore, metal nanoparticles can be generated in these systems by using reducing metal salts with reducing reagents, such as sodium borohydride (NaBH4), or citrate [24, 267]. Silver nanoparticles are also generated with the microreactor synthesis method [170, 171]. Another approach for synthesis of metal nanoparticle in microreactor is droplet-based processes.
Reagents in aqueous solutions are delivered separately though different inlets before being contacted in the microreactor [24, 268]. Different metal nanoparticles such as gold, copper, cobalt and palladium are generated by microfluidic systems [170, 171, 269].

*Oxides:*

Oxides nanoparticles are attractive because they can be used for many different applications [22]. As an example, the synthesis of iron oxide nanoparticles has attracted much attention, motivated by their wide range of applications. Colloidal iron oxides are used as single bielements in high density magnetic data storage arrays, ionic ferrofluids, and in the biomedical field, for example as contrast enhancement agents for Magnetic Resonance Imaging, or for hyperthermia [173]. The synthesis of oxides nanoparticles can be done by two common ways either by using either sol–gel or redox reactions [270, 271]. Two main strategies are pursued: (i) generating nanoparticles using microchannels or droplets as nanolitre well-stirred reactors, targeting nanosized particles but without advanced control over their shape, and (ii) synthesizing microstructures from the size and shape of droplets, taking advantage of the complex flow fields [24, 172, 173]. Silicalite nanoparticles have also been synthesized in a microreactor system [174, 176].

*Synthesis of crystalline semiconductor nanoparticles:*

The large surface area and tunable size- and shape-dependent properties of nanocrystals make them ideal materials for bioimaging, therapeutics, catalysis, and optoelectronics [272]. Microfluidic reactors have emerged as an attractive technology for nanocrystal synthesis as they offer far higher levels of control than can be achieved in conventional macroscale batch reactions [272]. One important class of crystalline semiconductor nanoparticles are quantum dots. Quantum dots (QDs)—spherically shaped compound semiconductor nanoparticles which exhibit size-dependent quantum confinement effects has been generated by this method. The microfluidic technique has
been successfully applied to the synthesis of numerous compound semiconductor nanoparticles—including CdSe, CdS, and InP, together with more complicated core/shell structures such as CdSe/ZnS, Fe2O3/SiO2 and CdSe/ZnSe/ZnS [175, 273-281].

**Combinatorial synthesis of nanoparticles and engineering multifunctional nanoparticles:**

By combining multi steps synthesis in microreactors a very complex and sophisticated nanostructures and nanoparticles can be produced [23]. The shape and surface properties of these new nanoparticles can be modified and changed simultaneously [22, 23]. A microfluidic platform for the rapid, combinatorial synthesis and optimization of nanoparticles has been developed [23]. It has also been demonstrated that many different formulations of poly(lactic-co-glycolic acid)-b-poly(ethylene glycol) nanoparticles of different size and surface composition can be synthesized [23].

The development of smart multifunctional targeted nanoparticles (NPs) that can deliver drugs at a sustained rate to specific cells and carry nanoscale imaging agents may provide better efficacy, lower toxicity, and enhanced prognosis for treatment of multiple diseases [181, 282]. Combining organic and inorganic materials synthesis strategies in microdevices opens opportunities for realizing hybrid microparticles or nanoparticles made of organic materials (microgels, microcapsules, nanogels, nanocapsules) and incorporating inorganic nanoparticles such as magnetic iron oxide or QDs. Such structures are of particular interest for bio imaging [24, 177, 179].
5.3 FLOW FOCUSING MICROFLUIDIC DEVICE

Flow focusing microfluidic device squeezes the solvent stream between two anti-solvent streams, resulting in rapid solvent exchange via diffusion [22]. Advanced microchannel geometries result in complex folding of fluid flows, which can completely mix two or more streams in a short period of time [22]. The implementation of these mixing techniques for the formation of nanoparticles in continuous flow has resulted in generation of nanoparticles with tunable nanoparticle size, and narrower size distribution, relative to those made with conventional bulk techniques [22]. Furthermore, microfluidic devices are able to rapidly mix reagents [122], provide homogenous reaction environments, control reaction temperature, and allow addition of reagents at precise time intervals. These features make the system suitable for nanoparticle synthesis [12]. Moreover, the simplicity and reproducibility of microfluidic device fabrication and handling small volumes with less expensive materials makes it an ideal platform for rapid synthesis and optimization of nanoparticles. In addition, by recent development in the field it is possible to carry out in-line characterization, feedback control, and continuous synthesis of nanoparticles that will contribute to the optimization and screening of libraries of nanoparticles.

![Figure 5.3](image)

**Figure 5.3** Color microscopic image demonstrating flow focusing ability of microfluidic channels by two emerging fluid streams. Food coloring dye was used for visualization purposes.
Figure 5.3 represents the flow focusing images obtained by color imaging of microfluidic channels. McCormick food and egg coloring dye was injected to the microfluidic channels to show flow focusing ability of microfluidic channels and for visualization purposes. Figure 5.4 represents two microfluidic devices with flow focusing capability. In the following two sections, the generation of liposomes and gold nanoparticles using flow focusing microfluidic technique have been demonstrated.

**5.3.1 Liposome Nanoparticle Generation**

Phosphatidylcholine, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, USA). Cholesterol (Ch) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco’s phosphate buffered saline (DPBS) was obtained from GIBCO. Stock solution of the phospholipids was prepared and was stored at $-20\,^\circ\mathrm{C}$. 
Nanoliposomes were prepared by injecting a lipid mixture into the center channel of the microfluidic device and phosphate buffered saline (with different concentration) was injected to the other microchannels. Schematic presentation of device setup is shown in Figure 5.5. The PHD 2000 Infuse/Withdraw syringe pump was used to introduce different mixtures with different flow rates to the microfluidic device. The variation of the flow rate and mixture ratio of reagents will influence the formation of nanoparticles. Zeiss Microscope that is equipped with a stage, and software was used for image acquisition and analysis. Dynamic light scattering (DLS) measurements was performed by employing 90Plus zeta sizer for measuring the hydrodynamic diameter of the liposome nanoparticles. This procedure can be done by dilution of the sample in 3 ml cuvette. Obtained liposomal nanoparticles were analyzed by DLS measurement.

Flow focusing in microfluidic device allows for rapid mixing of reagents in the microchannels in a controlled manner. In our experiments, it was observed that by keeping the central channel flow rate constant and increasing the side microchannel flow rates. The length of flow focusing region

Figure 5.5 Schematic presentation of microfluidic flow focusing device for nanoparticle generation with feeding microchannels and obtained liposome shown in the outlet (Inset: optical microscopy image of the microchannels, scale bar 50 µm).
(the dashed line in Figure 5.3) will decrease. This implies that by increasing the side channel flow rate, the central channel flow stream will be squished harder. Therefore, the central stream width will decrease. Moreover, for nanoparticle liposome generation, DPPC with flow rate of 50 μL/min in central channel and Dulbecco’s phosphate buffered saline with flow rate of 300 μL/min in side channels was used. After collecting the nanoliposome samples from the outlet of microfluidic device, DLS measurements were performed five times for our samples. However, only three of these runs are presented for brevity. Table 5.1 represents the DLS measurement results for the obtained liposomal nanoparticle sample. The average effective diameter was 354 (nm) and polydispersity was 0.308. The flow rate in the side microchannels was varied and by keeping the central channel flow rate constant. Therefore, the flow rate of side channels was decreased to 200 μL/min. It was observed that by increasing the flow rate in side channel, smaller nanoparticles will be obtained by keeping all the variables constant [291, 295].

Table 5.1- DLS measurement result for Liposome nanoparticles

<table>
<thead>
<tr>
<th>Run</th>
<th>Nanoparticle size and Polydispersity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Effec. Diameter(nm)</td>
</tr>
<tr>
<td>1</td>
<td>319</td>
</tr>
<tr>
<td>2</td>
<td>346</td>
</tr>
<tr>
<td>3</td>
<td>376</td>
</tr>
</tbody>
</table>
5.3.2 Gold Nanoparticle Generation

Synthesis of Gold nanoparticles (GNPs) were made through the reaction of chloroauric acid (HAuCl4) and sodium borohydride (NaBH4). GNPs were prepared by injecting chloroauric acid (HAuCl4) into the center channel of the microfluidic device and sodium borohydride (NaBH4) as reducing agent were injected into the two oblique side microchannels intersecting with the central microchannel at room temperature. The mixing of reactants was obtained in a flow focusing microfluidic device as presented in Figure 5.3. An aqueous solution of 26 mM sodium borohydride (NaBH4) was prepared before reaction by dissolving 5 mg NaBH4 (Aldrich, 99.99%) in 5 mL DI water. The obtained solution was further diluted for obtaining lower concentrations including 1mM solution. The PHD 2000 Infuse/Withdraw syringe pump was used to introduce different mixtures with different flow rates to the microfluidic device. Zeiss Microscope that is equipped with a stage, and software was used for image acquisition and analysis. Dynamic light scattering (DLS) measurements was performed by employing 90Plus zeta sizer for measuring the hydrodynamic diameter of the Gold nanoparticles. An aqueous solution of

![Image of HAuCl4 and Sodium Borohydride solutions](image_url)

**Figure 5.6** Aqueous solution of chloroauric acid (HAuCl4) and sodium borohydride (NaBH4)
chloroauric acid (HAuCl₄) and sodium borohydride (NaBH₄) are presented in Figure 5.6. For our purpose, the 1mM chloroauric acid (HAuCl₄) solution with flow rate of 50 μL/min was injected into center microchannel and sodium borohydride (NaBH₄) solution as reducing agent with flow rates of 100, 150, and 200 μL/min into two oblique side microchannels. Gold nanoparticles were generated by changing the flow rate at the inlet of microfluidic device. The obtained gold nanoparticles are presented in Figure 5.7. Figure 5.7A, B, and C corresponds to the flow rates of 100, 150, and 200 μL/min of sodium borohydride (NaBH₄) solution into two oblique side microchannels, respectively. After collecting the gold nanoparticles samples from the outlet of microfluidic device, DLS measurements were performed ten times for each sample. Here, only three of these measurements are presented for brevity. Table 5.2 represents the DLS data obtained for size and polydispersity of gold nanoparticles produced at flow rates of 100 μL/min of sodium borohydride (NaBH₄). Table 5.3 represents the DLS measurement obtained for size and
polydispersity of gold nanoparticles produced at flow rates of 150 μL/min of sodium borohydride (NaBH4).

Table 5.2- DLS data obtained for size and polydispersity of gold nanoparticles produced at flow rates of 100 μL/min of sodium borohydride (NaBH4)

<table>
<thead>
<tr>
<th>Run</th>
<th>Nanoparticle size and Polydispersity</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Effec. Diameter(nm)</td>
<td>Half width(nm)</td>
</tr>
<tr>
<td>1</td>
<td>107.2</td>
<td>63.7</td>
</tr>
<tr>
<td>2</td>
<td>106.9</td>
<td>64</td>
</tr>
<tr>
<td>3</td>
<td>105.1</td>
<td>63.1</td>
</tr>
</tbody>
</table>

Table 5.3- DLS data obtained for size and polydispersity of gold nanoparticles produced at flow rates of 150 μL/min of sodium borohydride (NaBH4)

<table>
<thead>
<tr>
<th>Run</th>
<th>Nanoparticle size and Polydispersity</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Effec. Diameter(nm)</td>
<td>Half width(nm)</td>
</tr>
<tr>
<td>1</td>
<td>132.4</td>
<td>65.7</td>
</tr>
<tr>
<td>2</td>
<td>129.1</td>
<td>69.9</td>
</tr>
<tr>
<td>3</td>
<td>131.4</td>
<td>66.1</td>
</tr>
</tbody>
</table>
Table 5.4 represents the DLS measurement obtained for size and polydispersity of gold nanoparticles produced at flow rates of 200 μL/min of sodium borohydride (NaBH4). Table 5.5 represents the average effective diameter (nm) and polydispersity of gold nanoparticles produced at the flow rates of 100, 150, and 200 μL/min of sodium borohydride (NaBH4) solution into two oblique side microchannels.

**Table 5.4-** DLS data obtained for size and polydispersity of gold nanoparticles produced at flow rates of 200 μl/min of sodium borohydride (NaBH4)

<table>
<thead>
<tr>
<th>Run</th>
<th>Nanoparticle size and Polydispersity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Effec. Diameter(nm)</td>
</tr>
<tr>
<td>1</td>
<td>105.2</td>
</tr>
<tr>
<td>2</td>
<td>105.1</td>
</tr>
<tr>
<td>3</td>
<td>101.9</td>
</tr>
</tbody>
</table>

**Table 5.5-** The average effective diameter (nm) and polydispersity of gold nanoparticles produced at the flow rates of 100, 150, and 200 μL/min of sodium borohydride (NaBH4) solution into side microchannels

<table>
<thead>
<tr>
<th>Flow Rate (μl/min)</th>
<th>Nanoparticle size and Polydispersity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Effec. Diameter(nm)</td>
</tr>
<tr>
<td>100</td>
<td>105</td>
</tr>
<tr>
<td>150</td>
<td>130.2</td>
</tr>
<tr>
<td>200</td>
<td>101.7</td>
</tr>
</tbody>
</table>
The average effective diameter of gold nanoparticles produced at the flow rates of 100, 150, and 200 μL/min of NaBH4 solution were 105, 130.2, and 101.7 nm, respectively. The average polydispersity of gold nanoparticles generated at the flow rates of 100, 150, and 200 μL/min of NaBH4 solution were 0.352, 0.26, and 0.248, respectively. This indicates that the variation of the flow rate ratio of reagents will influence the formation of nanoparticles. Furthermore, it should be noted that by changing the flow rate ratio of the side microchannels to the center microchannel the magnitude of the shear stress increases. Thus, it can be seen that by changing the flow rates in our flow focusing microfluidic device, the physical characteristics of the obtained gold nanoparticles can be facilely altered over the range of 100-133 nm.

5.4 CHAPTER SUMMARY

In this chapter, an overview of different methods for synthesis of nanoparticles was described. There are many different ways that nanoparticles can be synthesized. This depends on the type of the materials that will be used for synthesis. One possible way to categorize the NP synthesis is based on the fact that it is done in a bulk/bench-top manner or microfluidic manner. Microfluidic devices are able to rapidly mix reagents, and provide homogenous reaction environments. Moreover, the simplicity and reproducibility of microfluidic device fabrication and handling small volumes with less expensive materials makes it an ideal platform for rapid synthesis and optimization of nanoparticles. In particular, the flow focusing microfluidic devices was examined. Flow focusing microfluidic device squeezes the solvent stream between two anti-solvent streams, resulting in rapid solvent exchange via diffusion. Two different nanoparticles, namely, gold and liposomes were generated by using flow focusing microfluidic technique. Our microfluidic device
will provide a platform for continuous synthesis of gold and liposomal nanoparticles with rapid synthesis capabilities. The effect of the variation of the flow rate on the liposomal and gold nanoparticle size distribution was shown. In case of liposomal nanoparticles, it was shown that by increasing the flow rate in side channel, smaller nanoparticles can be obtained by keeping all the variables constant. Furthermore, it was shown that by changing the flow rates in our flow focusing microfluidic device, the size of the obtained gold nanoparticles can be facilely altered.
CHAPTER 6

CONCLUDING REMARKS AND FUTURE DIRECTIONS

6.1 CONCLUDING REMARKS

In this dissertation, an integrated microfluidic systems with shear-protective regions that enables cell and particle immobilization, sensor integration, and nanoparticle synthesis was presented. A novel and simple microfluidic platform for capturing small volumes of cells by using sidewall microgrooves and microposts was proposed. The cell docking patterns in the sidewall microgroove containing channels were studied. Both numerical and experimental investigations were performed within sidewall microgroove containing channels with three different widths (i.e., 50, 100, and 200 µm). It was observed that sidewall microgroove containing channels plays an important role in regulating cell positioning and patterning. Obtained results revealed that 10 to 14 cells were positioned inside sidewall channels with 200 µm in width, 2 to 5 cells were positioned within 100 µm wide, and 1 to 2 individual cells were docked within sidewall channel with 50 µm in width. In another word, it was demonstrated that microgroove size plays a key role in cell capturing. Particle modeling showed the prediction of cell positioning within sidewall microgrooves. The positions of cells docked within microgroove containing channels were also quantified. Furthermore, the shear stress variation and cell positioning in the sidewall microgrooves were correlated. The effect of channel width variation on the cell penetration was also investigated. Moreover, the histograms of cell locations in the microgrooves were provided and the most probable destination of the cells
was shown. In addition, our proposed platform has the capability of both integration of polymer coated piezoelectric microcantilevers, and capability of nanoparticle synthesis.

Thus, a unique polymer-coated piezoelectrically (ZnO) driven micro/nanomechanical cantilever for chemical vapor detection was devised and presented. Both the experimental evidence and theoretical rational of analyte vapor detection were presented. Two different polymers, namely, aromatic epoxy resin (DEN 431) and Fluoropel polymer (PFC) were used for coating of microcantilevers. Ethanol was detected as the analyte of choice. The mechanical quality factor of polymer-coated microcantilever-based sensors in the experiments were evaluated and illustrated to be in the range of 30 to 120. The details of mathematical modeling of piezoelectrically-driven micro/nanomechanical cantilever used in the experiments were also provided. Moreover, the change of resonance frequency of each polymer coated micro/nanomechanical cantilever to precisely asses the amount of increase or decrease in resonance frequencies were investigated.

Nanogram detection of chemical analyte (ethanol) by measuring added mass to the surface of polymer-coated piezoelectric microcantilever beam was obtained. To further validate our measurements, the amount of polymer (PFC) added to the surface of microcantilever by image analysis method and frequency shift method were calculated. To facilitate this objective, a MATLAB code was devised to detect the edges of the PFC coated microcantilever images by using Sobel edge-detection technique. In addition, the details of integration of microfluidic platform containing polymer coated piezoelectric microcantilever was provided. This integrated microdevice is composed of two parts, the first part is the microfluidic component. The second part is the self-sensing piezoelectric microcantilever component. Both three-dimensional (3D) printing and conventional soft lithography methods for developing of the microfluidic component were utilized. 3D printing technology which is a form of additive manufacturing offers a great deal
of freedom for designing the device components and changing their features. In addition, alternative physical set-up of the integrated microfluidic platform containing microcantilever array was presented. Hence, it is possible to use this simple and adaptable proposed platform as an electronic nose or artificial nose to detect odors, gases, vapors and other chemical and biochemical agents. These demonstrations also suggest that the polymer-coated piezoelectrically driven micro/nanomechanical cantilevers are ideally suited for a sensitive detection of a wide range of analytes.

In the microfluidic device, further investigation on extracting cell information from image data was carried out. Hence, a cell segmentation technique was developed for cell counting and extracting the cell information from the microfluidic device. Generally, segmentation subdivides an image into its constituent regions or objects. The level to which the subdivision is carried depends on the problem being solved. That is, segmentation should stop when the objects of interests in an application have been isolated. The selection of cell segmentation techniques is based on the application in hand. Our proposed algorithm were implemented to start with the selection of region of interest (ROI) by user input and extraction of features for image segmentation. Hence, at the beginning of the algorithm user was prompted to select region of interest which in our case was a groove that was located in the microfluidic device. Consequently, the sub-images (sub-grooves) were extracted and used for segmentation. Since the size of the microgrooves and number of sub-grooves were different in the obtained optical images, the user input has generated an adaptive technique which worked for all groove sizes. Consequently, corresponding black image with the same size of sub-grove were created by the proposed algorithm. The number of cells calculated from the proposed algorithm was compared with the number of cells that manually counted. The image segmentation method was performed over
ninety seven images. It was noted that the obtained results of cell counting was the same as data obtained from manual cell counting. However, there were difference between number of cells from the proposed technique and number of cells counted manually in some cases. The reason of this difference was partial location of the cells in the extracted sub-groove borders or the small size of the cells with very low intensity which more likely related to cells that were not alive. It was also due to agglomeration of cells together. Furthermore, all of the coordinates of cell location has been extracted for further fluidic analysis.

In this dissertation, capability of nanoparticle synthesis by our microfluidic platform was also explored. Microfluidic devices can provide a rapid and homogenous reaction environment with precise control over reagent addition. These features make them an ideal platform for nanoparticle synthesis. First, the concept of flow focusing microfluidic device was demonstrated. Two different nanoparticles, namely, gold and liposomes were generated by using flow focusing microfluidic technique. Our microfluidic device will provide a platform for continuous synthesis of gold and liposomal nanoparticles with rapid synthesis capabilities. Dynamic light scattering (DLS) measurements was performed for measuring the hydrodynamic diameter of the obtained nanoparticles. The effect of the variation of the flow rate on the liposomal and gold nanoparticle size distribution was shown. In case of liposomal nanoparticles, it was shown that by increasing the flow rate in side channel, one can obtain smaller nanoparticles by keeping all the variables constant. Furthermore, it was shown that by changing the flow rates in our flow focusing microfluidic device, the size of the obtained gold nanoparticles can be facilely altered.


6.2 FUTURE DIRECTIONS

There are some improvements that can be pursued for further investigation on the presented integrated platform. Below are some options and suggestions for the future research directions.

6.2.1 Investigation on different geometries of microgrooves in microfluidic setup:
In Chapter 2, the numerical and experimental observations obtained for square shape sidewall microgrooves were described. It is possible to expand the investigation on different shapes and angles of sidewall microgrooves (such as 60, 30 degree angle). Furthermore, one might consider to fabricate the microgrooves with the sizes different than those presented in Chapter 2 (such as 25, 300, and 400 µm).

6.2.2 Investigation on different cell types:
As described in Chapter 2 of this dissertation, our experiments was performed on NIH 3T3 mouse fibroblasts. Our microfluidic platform can and should be used for capturing of other cell lines as well. Therefore, it is possible to further investigate on the capturing of different cell lines (such as cancer cell lines or stem cells).

6.2.3 Investigation on embedded microcantilevers with different shapes and sizes:
In Chapter 3, the experimental and numerical observations obtained for rectangular shape microcantilever were described. It is possible to expand the investigation on different geometries such as triangular, trapezoidal and complex geometry shaped microcantilevers. As it was presented in Figure 3.23 of Chapter 3, one might consider to fabricate the microcantilevers with smaller sizes.
6.2.4 Investigation on embedded microcantilever arrays:

Another area for potential future work is to embed microcantilever arrays in the microfluidic platform. A physical set-up of the integrated microfluidic platform containing microcantilever array was presented in Figure 3.24. In addition, it is possible to coat each branch of the microcantilever array with different polymer. This will facilitate the detection of mixture of chemical analytes introduced into the integrated device.

6.2.5 Investigation on different analyte detection:

Our polymer coated microcantilever platform can and should be used for the detection of different analytes. Therefore, another area for potential future work is to detect analytes such as methanol, toluene, and 1-propanol.

6.2.6 Investigation on synthesis of different nanoparticles for coatings:

In Chapter 5, the synthesis of nanoparticles (i.e. gold) by our flow focusing microfluidic platform were described. It is possible to expand the investigation on synthesis of different nanoparticles (such as polymeric nanoparticles) which can be used for the coating of microcantilevers.
CHAPTER 7

REFERENCES


[33] A. Boisen, T. Thundat, 2009, Design and fabrication of cantilever array biosensors, Materials Today, 12, 9, 32-38, ISSN 1369-7021.


A. Sane, M.C. Thies, 2007, Effect of material properties and processing conditions on RESS of poly(l-lactide), The Journal of Supercritical Fluids, 40, 134–143.


APPENDIX A

LID DRIVEN CAVITY FLOW MODELING BY USING FINITE ELEMENT METHOD

A.1 INTRODUCTION
The extensive application of computational methods has become a prominent and efficient approach to the solution of many fluid dynamics problems. The applications of CFD is seen in many different industries, e.g., automobile, pharmaceutical, electronics, Micro Electro Mechanical Systems (MEMS), bioengineering, metallurgical casting, oil, and chemical processing, to name a few. The laminar incompressible flow in a square cavity whose top wall moves with a uniform velocity in its own plane has served over and over again as a model problem for testing and evaluating numerical techniques [51-53,56-57,60-65, 285-288]. Here, the finite element modeling of steady state, incompressible lid driven cavity flow governed by Navier-Stokes equations [282-284] will be considered.

A.2 PROBLEM STATEMENT
The problem setup for the lid driven cavity flow is shown in Figure A.1. An isothermal fluid in a square cavity was considered for modeling. The lid of the cavity is moved with a constant velocity of one, and no-slip boundary conditions are imposed on the walls. Consider a two-dimensional solution domain $\Omega$. The Navier-Stokes equations for incompressible laminar flow can be written in the following form.

$$\rho \left( u_n \frac{\partial u}{\partial x} + v_n \frac{\partial u}{\partial y} \right) = \frac{\partial (\sigma_{x} - P)}{\partial x} + \frac{\partial (\tau_{xy})}{\partial y} \quad (A.1)$$

$$\rho \left( v_n \frac{\partial v}{\partial x} + u_n \frac{\partial v}{\partial y} \right) = \frac{\partial (\tau_{xy})}{\partial x} + \frac{\partial (\sigma_{y} - P)}{\partial y} \quad (A.2)$$
where $\sigma_x$, $\sigma_y$, and $\tau_{xy}$ can be obtained by:

$$
\tau_{xy} = \tau_{yx} = \mu \left( \frac{\partial u}{\partial x} + \frac{\partial v}{\partial y} \right) \tag{A.3}
$$

$$
\sigma_x = -P - \frac{2}{3} \mu \left( \frac{\partial u}{\partial x} + \frac{\partial v}{\partial y} \right) + 2\mu \frac{\partial u}{\partial x} \tag{A.4}
$$

$$
\sigma_y = -P - \frac{2}{3} \mu \left( \frac{\partial u}{\partial x} + \frac{\partial v}{\partial y} \right) + 2\mu \frac{\partial v}{\partial y} \tag{A.5}
$$

$$
\begin{align*}
\left( u \frac{\partial u}{\partial x} + v \frac{\partial u}{\partial y} \right) &= \frac{1}{\rho} F_x - \frac{1}{\rho} \frac{\partial P}{\partial x} + \frac{1}{3} \mu \left( \frac{\partial u}{\partial x} + \frac{\partial v}{\partial y} \right) + \frac{\mu}{\rho} \left( \frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2} \right) \tag{A.6} \\
\left( u \frac{\partial v}{\partial x} + v \frac{\partial v}{\partial y} \right) &= \frac{1}{\rho} F_y - \frac{1}{\rho} \frac{\partial P}{\partial y} + \frac{1}{3} \mu \left( \frac{\partial u}{\partial x} + \frac{\partial v}{\partial y} \right) + \frac{\mu}{\rho} \left( \frac{\partial^2 v}{\partial x^2} + \frac{\partial^2 v}{\partial y^2} \right) \tag{A.7}
\end{align*}
$$

and because of incompressibility (from the continuity equation) one can simplify the above equations to:

$$
\begin{align*}
\left( u \frac{\partial u}{\partial x} + v \frac{\partial u}{\partial y} \right) &= \frac{1}{\rho} F_x - \frac{1}{\rho} \frac{\partial P}{\partial x} + \frac{\mu}{\rho} \left( \frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2} \right) \tag{A.8} \\
\left( u \frac{\partial v}{\partial x} + v \frac{\partial v}{\partial y} \right) &= \frac{1}{\rho} F_y - \frac{1}{\rho} \frac{\partial P}{\partial y} + \frac{\mu}{\rho} \left( \frac{\partial^2 v}{\partial x^2} + \frac{\partial^2 v}{\partial y^2} \right) \tag{A.9}
\end{align*}
$$

where $\rho$ is the density of fluid, $P$ is the pressure, and $u$, $v$ are the flow velocity components. Now, the momentum and continuity equations for specified boundary conditions will be solved.

### A.3 Finite Element Formulation

There are many different finite element formulations for solving the viscous incompressible laminar flow [282-284]. The velocity-pressure formulation is presented here. The method of weighted residuals with Galerkin’s criterion was applied [282-284]. For this purpose consider a two-dimensional flow domain $\Omega$ bounded by curve $\Gamma$. For a general element of this domain, $u$, $v$, and $P$ were selected as nodal variables and interpolate these variables in the form of $u = NU$, $v = NV$, and $P = N_pP$. $N$ is the interpolation function for $u$ and $v$ velocity, and $N_p$ is the
interpolation function for pressure. Since Galerkin method was used, the interpolation functions and weighting functions are the same.

\[
\left( u \frac{\partial N}{\partial x} + v \frac{\partial N}{\partial y} \right) = \frac{1}{\rho} \frac{\partial N}{\partial x} P - \frac{\mu}{\rho} \frac{\partial^2 N}{\partial x^2} u - \frac{\mu}{\rho} \frac{\partial^2 N}{\partial y^2} u
\] (A.10)

\[
\left( v \frac{\partial N}{\partial x} + u \frac{\partial N}{\partial y} \right) = \frac{1}{\rho} \frac{\partial N}{\partial y} P - \frac{\mu}{\rho} \frac{\partial^2 N}{\partial x^2} v - \frac{\mu}{\rho} \frac{\partial^2 N}{\partial y^2} v
\] (A.11)

Multiplying equations (A.10) and (A.11) by the weighting functions and integrating yields:

\[
\int \int N u \frac{\partial N}{\partial x} dx \; dy + \int \int N v \frac{\partial N}{\partial y} dx \; dy = \frac{1}{\rho} \int \int N \frac{\partial N}{\partial x} P \; dx \; dy - \frac{\mu}{\rho} \int \int N \frac{\partial^2 N}{\partial x^2} u \; dx \; dy - \frac{\mu}{\rho} \int \int N \frac{\partial^2 N}{\partial y^2} u \; dx \; dy
\] (A.12)

\[
\int \int N v \frac{\partial N}{\partial x} dx \; dy + \int \int N u \frac{\partial N}{\partial y} dx \; dy = \frac{1}{\rho} \int \int N \frac{\partial N}{\partial y} P \; dx \; dy - \frac{\mu}{\rho} \int \int N \frac{\partial^2 N}{\partial x^2} v \; dx \; dy - \frac{\mu}{\rho} \int \int N \frac{\partial^2 N}{\partial y^2} v \; dx \; dy
\] (A.13)

Integrating products by parts where necessary and neglecting resulting contour integrals gives:

\[
\int \int N u \frac{\partial N}{\partial x} dx \; dy u + \int \int N v \frac{\partial N}{\partial y} dx \; dy u = \frac{1}{\rho} \int \int N \frac{\partial N}{\partial x} P \; dx \; dy u - \frac{\mu}{\rho} \int \int N \frac{\partial^2 N}{\partial x^2} u \; dx \; dy u
\] (A.14)

\[
\int \int N u \frac{\partial N}{\partial x} dx \; dy v + \int \int N v \frac{\partial N}{\partial y} dx \; dy v = \frac{1}{\rho} \int \int N \frac{\partial N}{\partial y} P \; dx \; dy v - \frac{\mu}{\rho} \int \int N \frac{\partial^2 N}{\partial x^2} v \; dx \; dy v
\] (A.15)

The set of equations is completed by the continuity condition:

\[
\int \int N_p \left( \frac{\partial N}{\partial x} u + \frac{\partial N}{\partial y} v \right) dx \; dy
\] (A.16)

The Galerkin discretization applied to equation (A.1) and (A.2) can be combined with continuity equation. Therefore, a set of matrices will be obtained that can be written in submatrix form. These submatrices are:
\[ C_{11} = C_{33} = \iint \left( NU \frac{\partial N}{\partial x} + NV \frac{\partial N}{\partial y} + \mu \frac{\partial N}{\partial x} \frac{\partial N}{\partial x} + \frac{\mu}{\rho} \frac{\partial N}{\partial y} \frac{\partial N}{\partial y} \right) dx \, dy \quad (A.17) \]

\[ C_{12} = \iint \left( \frac{1}{\rho} N \frac{\partial N_P}{\partial x} \right) dx \, dy \quad (A.18) \]

\[ C_{13} = C_{31} = C_{22} = 0 \quad (A.19) \]

\[ C_{21} = \iint \left( N_P \frac{\partial N}{\partial x} \right) dx \, dy \quad (A.20) \]

\[ C_{23} = \iint \left( N_P \frac{\partial N}{\partial y} \right) dx \, dy \quad (A.21) \]

\[ C_{32} = \iint \left( \frac{1}{\rho} N \frac{\partial N_P}{\partial y} \right) dx \, dy \quad (A.22) \]

From these submatrices, it is possible to write the complete coefficient matrix as follows:

\[
\begin{bmatrix}
C_{11} & C_{12} & C_{13} \\
C_{21} & C_{22} & C_{23} \\
C_{31} & C_{32} & C_{33}
\end{bmatrix}
\begin{bmatrix}
u \\
p
\end{bmatrix} = \begin{bmatrix}
0 \\
0 \\
0
\end{bmatrix} \quad (A.23)
\]

**A.4 NUMERICAL SIMULATIONS**

Once the element matrices are assembled to form the system of algebraic equations and the boundary conditions are imposed, it is possible to solve these system of equations. Since, the steady state Navier-Stokes equations are nonlinear an iterative method can be used for solving this system.

**A.5 BOUNDARY CONDITIONS**

The boundary conditions applied to the solution domain is shown in Figure A.1. It should be noted that there is a discontinuity in boundary conditions at the upper corners of cavity. Two cases can happen: the upper corners are considered either as belonging to the upper moving side (leaky cavity), or they are assumed to belong to the fixed sides (non-leaky cavity). The leaky case which introduces singularity in the upper side of cavity was chosen.
A.6 SHAPE FUNCTIONS

As mentioned earlier, an eight node quadrilateral elements for velocities was employed for this study. The velocity shape functions are defined as follows:

\[ N_0 = -\frac{1}{4}(1 - \zeta)(1 - \eta)(1 + \eta + \zeta) \quad (A.24) \]
\[ N_1 = \frac{1}{4}(1 + \zeta)(1 - \eta)(\zeta - \eta - 1) \]
\[ N_2 = \frac{1}{4}(1 + \zeta)(1 + \eta)(\zeta + \eta - 1) \]
\[ N_3 = -\frac{1}{4}(1 - \zeta)(1 + \eta)(\zeta - \eta + 1) \]
\[ N_4 = \frac{1}{2}(1 - \zeta^2)(1 - \eta) \]
\[ N_5 = \frac{1}{2}(1 - \eta^2)(1 + \zeta) \]
\[ N_6 = \frac{1}{2}(1 - \zeta^2)(1 + \eta) \]
\[ N_7 = \frac{1}{2}(1 - \eta^2)(1 - \zeta) \]

The indexing starts from lower left corner of element and increases in clockwise direction in Figure A.2. The pressure shape functions are defined as follows:

\[ N_{P0} = \frac{1}{4}(1 - \zeta)(1 - \eta) \quad (A.25) \]
\[ N_{P1} = \frac{1}{4}(1 + \zeta)(1 - \eta) \]
\[ N_{P2} = \frac{1}{4}(1 + \zeta)(1 + \eta) \]
\[ N_{P3} = \frac{1}{4}(1 - \zeta)(1 + \eta) \]

Again, the indexing for pressure nodes starts from lower left corner of element and increases in clockwise direction.

A.7 QUADRILATERAL ELEMENTS
Figure A.2 represents the schematic of element chosen for the numerical modeling purpose. As shown in Figure A.2, an eight node quadrilateral elements (serendipity) for velocities coupled with a four node quadrilateral elements for pressure were employed. The velocities and pressures are located in nodes as presented in Figure A.2.

![Boundary conditions for lid driven cavity flow](image1)

**Figure A.1** Boundary conditions for lid driven cavity flow

![Schematic presentation of Quadrilateral element](image2)

**Figure A.2** Schematic presentation of Quadrilateral element

A.8 SOLUTION ALGORITHM
Read the input data from the file
Initialize arrays
  * Start iteration loop
    Initialize arrays to zero
    ** Loop over elements
      Relate the local node numbers to global node numbers
      Set the nodal values of velocity
      Initialize the $C$ submatrices
      - Start the Gaussian point integration
        Calculate velocity shape functions
        Derive $C_{11}$
        Calculate pressure shape functions
        Derive $C_{12}, C_{21}, C_{23}, C_{32}$
      - End of the Gaussian point integration
      Derive the coefficient matrix
      Assemble the unsymmetrical coefficient matrix
    ** End of Loop over elements
    Apply Boundary conditions for pressure and velocity
    Solve the equations
    Check the convergence criterion
  * End of iteration loop
• Echo the result

A.9 RESULTS

For modeling purposes, the low Reynolds number ($Re=100$) was considered. Figure A.3 presents the $u$-velocity distribution along the ($x=0.5$) line and $v$-velocity distribution along the ($y=0.5$) line. The computed results are compared with the grid-independent results of Ghia et al. [56]. Figures A.4, and A.5 represent the velocity, and streamline distribution in the solution domain for ($Re=100$).
Figure A.3 (Top) U-velocity comparison in lid driven cavity, (Bottom) V-velocity comparison in lid driven cavity

Figure A.4 Velocity distribution in lid driven cavity for 10x10 grid setup
A.10 DISCUSSION

The cavity chosen for this study was a square cavity with unit lengths (1x1). The solution domain was discretized with a uniform mesh of 10x10 elements, and results were obtained. As shown in Figure A.6, there are some differences in velocity distributions obtained by the present modeling and the Ghia et al. [56]. The reason for this discrepancy is due to advection effects. The Galerkin method is good in terms of predicting diffusion type problems. Therefore, upwinding effects should be considered to obtain a reliable solution. It should also be noted that advection will have stronger effect by increasing the Reynolds number.

The Galerkin finite element method leads to central approximations of the convective terms. Therefore is not optimal when convection dominate diffusion. In such cases, a stabilized finite element formulation for obtaining reliable numerical solutions should be used. A vortex was also observed in Figure A.5. The center of this vortex has moved toward the upper right corner of solution domain which is acceptable. If one consider the Stokes flow (flow with a very low
Reynolds number) the center of this vortex will be located in the symmetry line. However, for this case the center of the vortex moves away from the symmetry line.

A.11 SUMMARY

In this work, a steady-state two dimensional finite element modeling and coding were provided to investigate on lid driven cavity flow. In terms of numerical modeling, Galerkin method with velocity-pressure formulation was implemented. This approach provides simplicity for coding. Although the implementation of this approach is straightforward and simple, but it introduces some limitation in dealing with the flows with higher Reynolds numbers due to lack of upwinding considerations. Furthermore, the numerical results was compared against some benchmark solutions for validation purposes. The result was in a relatively good agreement with the benchmark results presented by Ghia et al. [56].

A.12 C++ SOURCE CODE

The finite element code for solving lid driven cavity flow is presented in the following:

```cpp
#include<iostream>
using namespace std;
#include<stdlib.h>
#include<assert.h>
#include<iomanip>
#include <fstream>
#include <cmath>
#include <stdio.h>
#include <string.h>

// Function Prototypes
void input_constants();
void input_arrays();
void create_arrays();
void create_array(double **&int, int, int);
void create_array(double *&, int);
void create_array(int *&int);
void create_array(int **&, int, int);
void delete_arrays();
void delete_array(double **&, int, int);
void delete_array(double *&int);
```
void delete_array(int *&, int);
void delete_array(int **&, int, int);
void create_arrays_1();
void delete_arrays_1();
nodal_freedom_matrix(int **, int);
int max(int **, int, int);
int max1(int *a1, int);
int min1(int *a2, int);
double max2(double *a3, int);
void shape(double **, double *);
void quad(int, int, double *, double *, double **, int *);
void transpose(double **a, int, int, double **);
void velocity_shape_func(double *, double **, int);
void pressure_shape_func(double *, double **, int);
void shape_der(double **, double **, int);
void shape_derf(double **, double **, int);
void multiply(double **a, double **c, double **b, int, int, int);
void stiffness(double **a, double **c, double **b, int, int, int);
void compacted(double **, double **, int *, int, int);
// Global variables
double **B, **a, **b, **c, **B1, **trans_derive, **B2, **B3, **C1,
**C2, **C3, **C4, **C5, **C6, **K_index, *z, **copy;
int *index, *num, **global_num, **global_index, *no, *checker, *node, **freedom;
double eps, visc, rho, det, ubar, vbar;
bool converged;
n int nband1, nband2, nxe1, nyel;
double templ;
int j;
double *Lx, *Ly, **c11, **c21, **c12, cte,
**c23, **c32, **coefficient, *RHS, *RHS_old,
**points, **coord, **pres_der_glob, *vel_shap_loc, **dummy,
**jacobian, **kay, **vel_der_loc, **vel_der_glob, **weights,
**pres_der_loc, **pres_shap_loc, **pressure_coord, **ke, **global_coord,
**vert_vel_shap_loc, **hor_vector1, **hor_vector2, *uvel, *vvel,
**vert_pres_shap_loc, **hor_vector_pres;
int i, k, iel, ntot, total_iter, known_values, iters, k10,
nel, nxe, nye, neg, nband, NoN, bond_n, nip, deg_freedom,
vel_shap_node, pres_shap_node, dImension;
main()
{
    input_constants();
    create_arrays();
    input_arrays();
nodal_freedom_matrix(freedom, bond_n);
    neg = max(freedom, NoN, deg_freedom);
    shape(points, weights);
    // Staring of loop over elements //
    nband = 0;
    for (iel = 0; iel < nel; iel++)
{ quad(iel,nxe,Lx,Ly,coord,num); 
k10=0;
    for(i=0;i<8;i++)
    {
        index[k10]=freedom[0][num[i]];
        k10=k10+1;
    }
    for(i=0;i<7;i=i+2)
    {
        index[k10]=freedom[1][num[i]];
        k10=k10+1;
    }
    for(i=0;i<8;i++)
    {
        index[k10]=freedom[2][num[i]];
        k10=k10+1;
    }
    for(i=0;i<9;i++)
    {
        global_num[i][iel]=num[i];
        create_array(B,dimension,vel_shap_node);
        for(int i20=0;i20<2;i20++)//initializing the B matrix
            for(int j20=0;j20<8;j20++)
                B[i20][j20]=0.0;
        transpose(coord,vel_shap_node,dimension,B);
        for(i=0;i<2;i++)
            for(int j=0;j<8;j++)
                global_coord[i][num[j]]=B[i][j];
        delete_array(B,dimension,vel_shap_node);
        for(i=0;i<ntot;i++)
            cout<<" index["<<i<<"]= "<<index[i]<<endl;
            for(i=0;i<ntot;i++)
                global_index[i][iel]=index[i];
            int max_gl=max1(index,ntot);
            int min_gl=min1(index,ntot);
            // cout<<"maximum index for element "+iel<<"=
            // cout<<"minimum index for element "+iel<<"=
            int bandwidth=max1(index,ntot)-min1(index,ntot);
            if(nband<bandwidth)
            {
                nband=bandwidth;
                cout<<"nband"<<nband<<endl;
            }
    } // End of loop over elements
    // delete_arrays();
    nband1=2*(nband+1)-1;
    nband2=nband+1;
    known_values=5;
    create_arrays_1();
    for(i=0;i<neq;i++)
    {
        RHS[i]=0.0;
        RHS_old[i]=0.0;
    }
    for(i=0;i<known_values;i++)
    {
        node[i]=i;
}
checker[i]=0;
cte[i]=1.0;

for(int iter=0;iter<total_iter;iter++)
{
    cout<<"iter number="<<iter<<endl;
    converged=0;
    for(int i10=0;i10<neq;i10++)
        for(int j10=0;j10<nband1;j10++)
            coefficient[i10][j10]=0.0;
    for(i10=0;i10<neq;i10++)
        for(int j10=0;j10<neq;j10++)
            K_index[i10][j10]=0.0;
    for(i10=0;i10<nband2;i10++)
        for(int j10=0;j10<neq;j10++)
            dummy[i10][j10]=0.0;
    for(i10=0;i10<ntot;i10++)
        for(int j10=0;j10<ntot;j10++)
            ke[i10][j10]=0.0;

    for(iel=0;iel<nel;iel++)
    {
        cout<<"element number="<<iel<<endl;
        for(i=0;i<num;i++)
            num[i]=global_num[i][iel];
        create_array(B,vel_shap_node,dimension);
        create_array(B1,dimension,vel_shap_node);
        for(i=0;i<num;i++)
            for(int j=0;j<num;j++)
                B1[i][j]=global_coord[i][num[j]];
        transpose(B1,dimension,vel_shap_node,B);

        for(i=0;i<num;i++)
            for(int j=0;j<num;j++)
                coord[i][j]=B[i][j];
        delete_array(B,vel_shap_node,dimension);
        delete_array(B1,dimension,vel_shap_node);
        for(i=0;i<ntot;i++)
        {
            index[i]=global_index[i][iel];
            //cout<<"index["<<i<<"]="<<index[i]<<endl;
        }

        pressure_coord[0][0]=coord[0][0];
        pressure_coord[1][0]=coord[2][0];
        pressure_coord[2][0]=coord[4][0];
        pressure_coord[3][0]=coord[6][0];
        pressure_coord[0][1]=coord[0][1];
        pressure_coord[1][1]=coord[2][1];
        pressure_coord[2][1]=coord[4][1];
        pressure_coord[3][1]=coord[6][1];
        for(i=0;i<vel_shap_node;i++)
            uvel[i]=(RHS[index[i]]+RHS_old[index[i]])*0.5;
        for(i=vel_shap_node; i<ntot; i++)
        {
            vvel[i-vel_shap_node]=
            pres_shap_node=(RHS[index[i]]+RHS_old[index[i]])*0.5;
        }
}
}
for(i=0;i<vel_shap_node;i++)
for(int j=0;j<vel_shap_node;j++)
c11[i][j]=0.0;
for(i=0;i<pres_shap_node;i++)
for(int j=0;j<pres_shap_node;j++)
c12[i][j]=0.0;
for(i=0;i<pres_shap_node;i++)
for(j=0;j<vel_shap_node;j++)
c21[i][j]=0.0;
for(i=0;i<pres_shap_node;i++)
for(j=0;j<vel_shap_node;j++)
c23[i][j]=0.0;
for(i=0;i<pres_shap_node;i++)
for(j=0;j<pres_shap_node;j++)
c32[i][j]=0.0;
// Gaussian integration
for(i=0;i<nlp;i++)
{
    cout<<"Gaussian integ. loop="<<i<<endl;
    // velocity effects
    velocity_shape_func(vel_shap_loc,points,i);
    for(int ik=0;ik<vel_shap_node;ik++)
        vert_vel_shap_loc[ik][0]=vel_shap_loc[ik][0];
        ubar=0.0;
    for(ik=0;ik<vel_shap_node;ik++)
        ubar=ubar+vel_shap_loc[ik]*uvel[ik];
        vbar=0.0;
    for(ik=0;ik<vel_shap_node;ik++)
        vbar=vbar+vel_shap_loc[ik]*vvel[ik];

    if(iter==0)
    {
        ubar=1.0;
        vbar=0.0;
    }

    jacobian[0][0]=0.0;jacobian[0][1]=0.0;jacobian[1][0]=0.0;jacobian[1][1]=0.0;
    shape_der(vel_der_loc,points,i);
    for(ik=0;ik<vel_shap_node;ik++)
    {
        jacobian[0][0]=jacobian[0][0]+vel_der_loc[0][ik]*coord[ik][0];
        jacobian[0][1]=jacobian[0][1]+vel_der_loc[0][ik]*coord[ik][1];
        jacobian[1][0]=jacobian[1][0]+vel_der_loc[1][ik]*coord[ik][0];
        jacobian[1][1]=jacobian[1][1]+vel_der_loc[1][ik]*coord[ik][1];
    }
    det=jacobian[0][0]*jacobian[1][1]-jacobian[0][1]*jacobian[1][0];
    if(det==0) det=0.0000001;
    //inverse of determinant/
    temp1=0.0;
    temp2=jacobian[0][0];
    jacobian[0][0]=jacobian[1][1];
    jacobian[1][1]=temp2;
    jacobian[0][1]=-jacobian[0][1];
    jacobian[1][0]=-jacobian[1][0];
    jacobian[0][0]=jacobian[0][0]/det;
    jacobian[1][1]=jacobian[1][1]/det;
    jacobian[0][1]=jacobian[0][1]/det;
vel_der_glob[ix][kx]=vel_der_glob[ix][kx]+jacobian[ix][jx]*vel_der_loc[jx][kx];

for(iz=0;iz<8;iz++)
{
    hor_vector1[0][iz]=vel_der_glob[0][iz];
    hor_vector2[0][iz]=vel_der_glob[1][iz];
}

create_array(transimizeBox,vl_shap_node,dimension);
transpose(vel_der_global, dimension, vel_shap_node, trans_derivative);
create_array(B2, vel_shap_node, dimension);
create_array(B3, vel_shap_node, vel_shap_node);
create_array(C1, vel_shap_node, vel_shap_node);
create_array(C2, vel_shap_node, vel_shap_node);

for(int i21=0;i21<vel_shap_node;i21++)
    for(int j21=0;j21<dimension;j21++)
        B2[i21][j21]=0.0;

for(int i22=0;i22<vel_shap_node;i22++)
    for(int j22=0;j22<vel_shap_node;j22++)
    {
        B3[i22][j22]=0.0;
        C1[i22][j22]=0.0;
        C2[i22][j22]=0.0;
    }

multiply(trans_derivative, vel_shap_node, dimension, dimension);
multiply(B2, vel_der_loo, B3, vel_shap_node, dimension, vel_shap_node);
multiply(vert_vel_shap_local, hor_vector1, C1, vel_shap_node, 1, vel_shap_node);
multiply(vert_vel_shap_local, hor_vector2, C2, vel_shap_node, 1, vel_shap_node);
delete_array(trans_derivative, vel_shap_node, dimension);

for(int i6=0;i6<vel_shap_node;i6++)
    for(int j6=0;j6<vel_shap_node;j6++)
    {
        c11[i6][j6]=c11[i6][j6]+B3[i6][j6]*det*weights[i]+C1[i6][j6]*det*weights[i]*ubar+C2[i6][j6]*det*weights[i]*vbar;
        // cout<<c11"<<i6"<<","<<j6"="<<c11[i6][j6]<<endl;
    }

// End of velocity effects//
delete_array(B2, vel_shap_node, dimension);
delete_array(B3, vel_shap_node, vel_shap_node);
delete_array(C1, vel_shap_node, vel_shap_node);
delete_array(C2, vel_shap_node, vel_shap_node);

/////////// pressure effects //////////
pressure_shape_func(pres_shap_loc, points, i);

for(ik=0;ik<pres_shap_node;ik++)
    vert_pres_shap_loc[ik][0]=pres_shap_loc[ik];
shape_derv(pres_der_loc, points, i);
for(ik=0;ik<pres_shap_node;ik++)

```c
{ jacobian[0][0]=jacobian[0][0]+pres_der_loc[0][ik]*pressure_coord[ik][0];
  jacobian[0][1]=jacobian[0][1]+pres_der_loc[0][ik]*pressure_coord[ik][1];
  jacobian[1][0]=jacobian[1][0]+pres_der_loc[1][ik]*pressure_coord[ik][0];
  jacobian[1][1]=jacobian[1][1]+pres_der_loc[1][ik]*pressure_coord[ik][1];
}
det=jacobian[0][0]*jacobian[1][1]-jacobian[0][1]*jacobian[1][0];
if(det==0) det=0.000001;
// inverse of determinent for pressure//
double temp1=0.0;
templ=jacobian[0][0];
jacobian[0][0]=jacobian[1][1];
jacobian[1][1]=templ;
jacobian[0][1]=-jacobian[0][1];
jacobian[1][0]=-jacobian[1][0];
jacobian[0][0]=jacobian[0][0]/det;
jacobian[1][1]=jacobian[1][1]/det;
jacobian[0][1]=jacobian[0][1]/det;
jacobian[1][0]=jacobian[1][0]/det;
// End of inverse of determinent//
for(iz=0;iz<2;iz++)
  for(int jz=0;jz<4;jz++)
    pres_der_glob[iz][jz]=0.0;
for(ix=0;ix<2;ix++)  //Multiply section
  for(int kx=0;kx<4;kx++)
    for(int jx=0;jx<2;jx++)
      pres_der_glob[ix][kx]=pres_der_glob[ix][kx]+jacobian[ix][jx]*pres_der_loc[jx][kx];
    for(iz=0;iz<4;iz++)
      hor_vector_pres[0][iz]=pres_der_glob[0][iz];
create_array(C3,vel_shap_node,vel_shap_node);
create_array(C4,vel_shap_node,vel_shap_node);
create_array(C5,pres_shap_node,vel_shap_node);
create_array(C6,pres_shap_node,vel_shap_node);
for(int i23=0;i23<vel_shap_node;i23++)
  for(int j23=0;j23<vel_shap_node;j23++)
    { C3[i23][j23]=0.0;
      C4[i23][j23]=0.0;
    }
for(int i24=0;i24<pres_shap_node;i24++)
  for(int j24=0;j24<vel_shap_node;j24++)
    { C5[i24][j24]=0.0;
      C6[i24][j24]=0.0;
    }
multiply(vert_vel_shap_loc,hor_vector_pres,C3,vel_shap_node,1,vel_shap_node);
for(int i7=0;i7<pres_shap_node;i7++)
  for(int j7=0;j7<pres_shap_node;j7++)
    c12[i7][j7]=c12[i7][j7]+C3[i7][j7]*det*weights[i]/rho;
for(iz=0;iz<4;iz++)
  hor_vector_pres[0][iz]=pres_der_glob[1][iz];
```
multiply(vert_vel_shap_loc, hor_vector_pres, C4, vel_shap_node, 1, vel_shap_node);
for (i7=0; i7<pres_shap_node; i7++)
    for (int j7=0; j7<pres_shap_node; j7++)
        c32[i7][j7] = c32[i7][j7] + C4[i7][j7] * det*weights[i]/rho;

multiply(vert_pres_shap_loc, hor_vector1, C5, pres_shap_node, 1, vel_shap_node);
multiply(vert_pres_shap_loc, hor_vector2, C6, pres_shap_node, 1, vel_shap_node);

for (i7=0; i7<pres_shap_node; i7++)
    for (int j7=0; j7<pres_shap_node; j7++)
        c21[i7][j7] = c21[i7][j7] + C5[i7][j7] * det*weights[i];

for (i7=0; i7<pres_shap_node; i7++)
    for (int j7=0; j7<pres_shap_node; j7++)
        c23[i7][j7] = c23[i7][j7] + C6[i7][j7] * det*weights[i];

delete_array(C3, vel_shap_node, vel_shap_node);
delete_array(C4, vel_shap_node, vel_shap_node);
delete_array(C5, pres_shap_node, vel_shap_node);
delete_array(C6, pres_shap_node, vel_shap_node);

// end of element stiffness matrix assembly

stiffness(ke, c11, c12, c21, c23, c32, vel_shap_node, pres_shap_node, ntot);
    compacted(coefficient, ke, index, nband, ntot);
    for (int i17=0; i17<neq; i17++)
    {
        for (int j17=0; j17<nband1; j17++)
            cout<<" coefficient"
            <<i17
            <<","<<j17
            <<"=
            <<coefficient[i17][j17]"<<endl;
    }

    // end of element stiffness matrix assembly

    // //////////////////////////////////////////////////////////////////////
    // Applying (B.C.) //////////////////////////////////////////////////////////////////////
    for (i=0; i<neq; i++)
        RHS[i] = 0.0;
    for (i=0; i<known_values; i++)
        no[i] = freedom[checker[i]][node[i]];
    for (i=0; i<known_values; i++)
    {
        coefficient[no[i]-1][nbond2] = coefficient[no[i]-1][nbond2] + 1.e20;
        RHS[no[i]-1][nbond2] = coefficient[no[i]-1][nbond2] * cte[i];
        // cout<< " RHS"<<no[i]-1<<"= " <<RHS[no[i]-1]<<endl;
    }

    // //////////////////////////////////////////////////////////////////////
    // Gaussian_elimination(coefficient, dummy, neq, nbond2);
    // solver(coefficient, dummy, RHS, neq, nbond2);
    // RHS[0]=0.0;
    // converged(RHS, RHS_old, eps, converged, neq);
    // if ((converged) || (iters==total_iter))
    //     break;
    cout<<" iter="""<<iter;
cout << "end of iteration" << endl;
} // end of iter loop

output();
delete_arrays();
delete_arrays_1();
return 0;
}
APPENDIX B

CONTRIBUTIONS

B.1 JOURNAL PAPERS


- **Khabiry, M., Faegh S., Jalili, N., Sridhar S.**, Piezoelectric polymer-coated microcantilevers for chemical sensing platform, In preparation


B.2 BOOK CHAPTER


B.3 PEER REVIEWED CONFERENCE PAPERS


B.4 POSTER AND PRESENTATIONS

- Khabiry M., Jalili N., Sridhar S., Gold nanoparticle generation with microfluidic device, Dolomite Ltd., 2013
- Khabiry M., Jalili N., Microcantilever coated with a hydrophobic polymer, Second MIE Engineering As Art competition 2014, Northeastern university

B.5 OTHER PUBLICATIONS (NOT RESULTED FROM THIS DISSERTATION)


