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Physics and Applications of a PDMS Based Centrifugal Microfluidic System

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PHYSICS AND APPLICATIONS OF A PDMS BASED CENTRIFUGAL MICROFLUIDIC SYSTEM

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

The Department of Mechanical Engineering

by

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B.S., Southeast University, 2005
M.S., Southeast University, 2008
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ABSTRACT

The objective of this research work is to develop a centrifugal microfluidic system for general purposes based on microfabrication technologies including SU-8 photolithography, polydimethylsiloxane (PDMS) casting.

The main contribution of this research is to integrate a flyball governor system into the polymer based centrifugal microfluidic platform. A series of function units are developed based on this unique mechanism. In the first part, three pinch valve systems were designed and tested. The first one is based on the magnetic force and the second one is on the basis of spring force and the last one is a membrane valve. All valving system demonstrate good control of the fluid movement. The latter two valves are capable of sequential control. It proves that the flyball governor system is very compatible with centrifugal fluidic technologies. The major advantage of this new actuation technology is that its burst frequency can be conveniently manipulated by adjusting the parameters of the mechanical system without changes in the fluidic pattern.

Next, two types of inward pumping systems were designed and tested. The result shows that both the inward pumps were capable of the pumping over a radial distance of 21mm in a short time. It thus improves the usage of space on the disc and paves the way to interconnect several functional units.

Then as a proof of concept, a sequential valving system capable of metering and centrifugal sediment was developed for plasma extraction from whole blood. The resulting residual cell concentration was less than 0.5%.

In the last part, a micromixer was developed based on the similar principle. The results show that the flyball governor system can effectively agitate the chaotic mixing of the sample liquids by periodically deflecting the PDMS membrane of the mixing chamber. The mixing effect can thus be enhanced.
This new design for the polymer-based centrifugal microfluidic system offers some major advantages over those reported in the field: simple fabrication, robust control and easy integration. It demonstrates great potential for complex fluid handling needed in biological, chemical and medical applications in Lab-on-CD systems.
CHAPTER 1. INTRODUCTION

The rapid developing technology of microelectromechanical systems (MEMS) has opened up new opportunities to develop novel micro-sized instruments. The microfluidic technology, a subarea of the MEMS field, has been used to manipulate fluids at the microscopic level and brought the benefits of miniaturization and low consumption of reagents and short analysis time, and meanwhile offered significantly enhanced sensitivity. The microfluidic system come with considerable advantages in these aspects which features a full process integration for all the required analysis steps such as liquid sample handling and manipulation (pumping and valves), extraction/separation, and high precision detections. It can reduce the consumption of sample and reagent and achieve a rapid response and high sensitivity. Therefore, it can be regarded as a prominent candidate to perform small-scale sample separations/extractions. Although significant efforts have been devoted to this area in recent years, most of these microfluidic systems do not show much advantages in terms of portability because they are limited to the external pumps or electrochemical method to introduce liquids into the system. As an important branch of the microfluidic system, the centrifugal microfluidic platform has gradually become the focus of academic and industrial area in the past decade. Recently, great efforts have been devoted to developing centrifugal microfluidic devices that enable miniaturized, parallel and autonomous analytical operations while significantly reducing the processing time, sample consumption and total cost. These devices are able to integrate fluid control systems such as pumps, valves and mixers with other functional units to into one compact disc. Their most attractive merit is to use the centrifugal force for flow control and therefore eliminate direct external connections such as syringe pump or high voltage power supply. As a result, centrifugal microfluidic can serve as a powerful tool for automation of biochemical assay and point-of-care applications.
This platform usually consists of two parts as shown in Figure 1.1: (1) a disposable CD that contains fluidic networks, and (2) a spin stand setup that includes a motor that drives the CD. From its inception, centrifugal microfluidics has attracted researchers because of the unique nature of fluid propulsion on a CD. Defined as centrifugal pumping, centrifugal forces induced by spinning the disc are used to propel liquids in the disc. The use of centrifugal pumping has primarily been applied to developing tools for biomedical applications, for example in blood panel analysis and immunoassay development. However, recent endeavors in centrifugal microfluidics have renewed interest in adapting CD based technology to create the next generation in vitro diagnostic systems. Physicians and engineers are particularly interested in developing integrated nucleic acid analysis systems for clinical diagnostics.

![Microfluidic Disc](image)

Figure 1.1 Schematic of a centrifugal microfluidic platform

1.1 History of the centrifugal microfluidics

The concept of the centrifugal microfluidic system was coined in the late 1960s by Anderson from Oak Ridge National Labs (ORNL) who fabricated a clinical analyzer based on a spinning rotor[1]. The device, called a centrifugal analyzer, utilized centrifugal forces in order to combine fluids that were subsequently examined by additional off-rotor optical instruments. The operation of the system was simple: the rotor was fabricated such that channels and risers (i.e., three-dimensional
barriers) along the radial axis kept sample and reagent aliquots separated during fluid loading. During spinning, the fluids were driven over the barriers into an optical cuvette positioned on the periphery of the rotor. As the fluids mixed and reactions started, absorbance changes were detected with a light source and a photomultiplier tube. The fluidic network was arrayed on the rotor such that after loading, multiple experiments could simultaneously be performed. Centrifugal analyzers were initially used for kinetic assay development. As time progressed additional optical technologies were incorporated to measure light transmittance, fluorescence, chemiluminescence, and light-scattering properties of reaction mixtures. The implementation of these functional units can be helpful for applications in chemistry, toxicology, immunology, and hematology. The system was also miniaturized in a portable setup. In this design, the fluidic networks were adapted to a more disc-like profile while the instrumentation was miniaturized and integrated for clearer automation.

Nowadays, there are several companies marketing instruments based on centrifugal microfluidic technologies. Abaxis develops a point of care blood analyzers to the medical market. The product is able to centrifuge and separate the plasma from blood, mix the diluted plasma, rehydrate the reagent beads, oversee dozens of enzymatic reactions at the same time and output the results[2]. The compact analyzer is about the size of a shoebox as shown in Figure 1.2. The operation can be completed in around 12 min. The Abaxis system represented a significant leap in the CD microfluidics by demonstrating that the integration of several processing steps could be realized on one disc.
Another Company named Gyros is a leading company in the immunoassays CD. Their product enables to deliver immunoassay data over a broad dynamic range, and save time, sample and reagents[3]. This is achieved through precise, automated control of centrifugal and capillary forces to steer liquid flow in proprietary nanoliter-scale microfluidic structures. Other companies have also developed centrifugal-based platforms for point-of-care diagnostics such as Focus Diagnostics, Quadraspec and Burstein Technologies.

Although centrifugal microfluidic technology has experienced extensive development in the fields of academia and industry, the market is still in short of commercial products today. The centrifugal
microfluidic technology demonstrates many advantages compared to other microfluidic platforms, but commercialized products still come across several barriers to hurdle the widespread use of microfluidic platforms. Any commercial microfluidic platform needs to bring advantages to the user in terms of cost, throughput, interface, and accuracy compared to the current methods.

1.2 Technology review for centrifugal microfluidic functions

This section presents a brief introduction to the major techniques used in the centrifugal microfluidic platform today. All the technologies are developed on the basis of the centrifugal force as the primary propulsion method.

1.2.1 Centrifugal pumping

In centrifugal microfluidic systems, the use of centrifugal force is a unique mechanism to drive the liquids radially outwards from the center to the edge of the disc’s the disc spins, the pressure gradient is exerted on the liquid. The average velocity $U$ of the liquid in a microchannel can be expressed as (see Figure 1.3) [4]

$$U = \frac{D_h \rho \omega^2 \bar{r} \Delta r}{32 \mu L}$$

Where $D_h$ is the hydraulic diameter of the channel, $\rho$ is the density of the liquid, $\omega$ is the angular velocity of the disc, $\bar{r}$ is the average distance of the liquid in the channels from the center of the disc, $\Delta r$ is the radial extent of the fluid, $\mu$ the viscosity of the fluid, and $L$ the length of the liquid in the microchannel.
1.2.2 Metering

Volume metering is another important function in centrifugal microfluidic. This process can define the certain volume of the fluidic sample or reagent in demand. Metering is usually achieved through the simple use of an overflow channel connected to a fluidic chamber. It can be realized by adding an overflow channel to the chamber. As the liquid reaches the level of the overflow channel, the unwanted fluid can be directed to a waste chamber while the selected liquid remains in the metering chamber for the following operations. The desired volume can be pre-defined by the size of the metering chamber and the position of the overflow chamber.

1.2.3 Valving

Valving is an essential function of any fluidic system, which enables the precise control of fluid movement. Various valves have been particularly proposed for centrifugal microfluidic devices. Among them, there are two widely used valves: the hydrophobic valve and capillary valve. The hydrophobic valve can be achieved by either hydrophobic patches or geometrical changes in the channel (Figure 1.4). The liquid can be eventually forced to pass the valve by increasing the rotational speed beyond the burst frequency. The capillary valve is based on the balance between
centrifugal pressure and surface tension in a hydrophilic material. All these valves as well as the siphon valve are limited to the surface tension of the liquid especially for the highly wetting liquids. To overcome the limitations of these passive valves, some sacrificial valves have also been reported such as wax valve[5], film valve[6] and dissolvable membrane valve[7]. They take advantage of the phase transition and can operate independently of the rotational speed or valving locations. However, the phase-change substance needs to be deposited in the microchannels, which adds complexity to the manufacturing process and the direct contact between the substance and the solution may cause cross-contamination.

Figure 1.5 Schematic of typical hydrophobic valves (a and b) and capillary valves(c)

1.3 The fabrication for the centrifugal microfluidics

There are generally two methods to fabricate of centrifugal microfluidics. One is based on CNC machine. The fluidic channels and chambers are directly engraved on plastic discs such as PMMA, PC and COC. The established centrifugal microfluidic system usually includes several layers of structures. All the layers are then bonded together with an adhesive membrane or tapes to set up a complete system. The feature size of this method is around 1mm. It usually demands high precision fabrication process. Another optional method is to use polymer-based photolithography technology, which is widely used in the conventional microfluidic system. It generally involves UV lithography of photoresist such as SU-8 and AZ, PDMS molding process,
micro cast with the PDMS intermediate mold, and PDMS to PDMS bonding. The detailed fabrication procedures are presented in the next several sections. The major advantage of this method is that the feature size of the fluidic system can go down to 5μm. Therefore, this method is more capable of integrated miniaturized system. In this work, we take advantages of the facilities in Center for Advanced Microstructures & Devices (Louisiana state university) and adopt this method to produce the centrifugal microfluidic devices used in this work.

1.3.1 UV Lithography of SU-8
SU-8 is a negative resist commonly used in the fabrication of high aspect ratio microstructures and devices. As a negative photoresist, when it is exposed to ultraviolet light, the exposed regions get cured and will remain after development while the unexposed areas are removed. It is also known for its excellent UV lithography properties and widely used in the fabrication of high aspect ratio microstructures and devices. In addition, the exposed (and cured) SU-8 polymer has excellent physical and chemical properties, and is highly resistant to most chemicals and very stable in high temperature. Because of these advantages, UV lithography of SU-8 is one of the key technologies used in fabricating the spilled oil detection system presented in this dissertation. To explain how the technology is used, we will use the fabrication of high aspect ratio micro-columns as the example here. The primary manufacturing process is as follows:

1) Spin-coat SU-8. SU-8 resist, especially the SU-8 50 and 100 series used in our work, has high viscosity, therefore flows very slowly once poured on the wafer. The exposed SU-8 resist can be easily removed with solvents such as acetone or SU-8 developer. The thickness of SU-8 resist depends on factors such as the viscosity of SU-8, spinning time and speed. The relationship curves between the resist thickness and these factors are readily found from the data sheets provided by the vendor (Microchem, USA)
2) Prebake. The purpose of prebake is to remove the solvent from the SU-8 resist. After prebaking, SU-8 becomes a solid layer on the wafer. The prebaking temperature has to be carefully controlled to avoid the residual stress and achieve lithography results. Generally, it is proportional to the thickness of the SU-8. For example, it takes 6 hours for a resist of 600μm and take 4 hours for resist with a thickness of 400μm. The ramping and dwelling times before and after the maximum temperature are chosen to make the smooth transition.

3) Exposure. Place the wafer into the UV station with the mask properly covered on it. During the exposure process, the ultraviolet (UV) light can only get through the transparent part of the mask and expose the SU-8 layer as expected. The exposure dosage increases with the thickness of the resist.

4) Post bake. When the SU-8 resist is exposed to UV light, an acid can be generated inside the resist, leading to the cross-link of the monomer. After the exposure, the exposed wafer coated with SU-8 needs to be post-baked to improve the cross-linking process, enhance the lithography quality, and minimize possible residual stress.

5) Development. After the post bake, the cross-link in SU-8 is almost completed, which means the SU-8 developer now can only remove the unexposed parts but not exposed parts. The development may take a longer time if the exposed wafer is placed in facing up position in the developer. When the wafer is placed in the “facing-down” position (with the SU-8 side facing down vertically), the gravity helps to enhance the development process by allowing the developed chemicals to drop off and improve the circulation. The developed wafer needs to be carefully and slowly taken out from the developer to prevent them from mechanical damages caused by the developer fluid.

6) Hard bake. After the development, the resulting SU-8 master is obtained. The master may be
heated for the third time and continue cross-link the resist. The temperature is usually higher than the soft baking and post-exposure baking. This process is optional and only necessary when the residue stress is significant.

![fabrication process of SU-8](image)

**Figure 1.6** The typical fabrication process of SU-8 to obtain the master mold

### 1.3.2 PDMS Casting

PDMS (Sylgard 184, Dow Corning, USA) can be used in a molding process to make an intermediate negative pattern, which comes from an SU-8 master mold. The master mold can be prepared by using SU-8 lithography process as introduced in the previous section.

The detailed steps of PDMS molding process are as follow:

1) Preparation of the PDMS mixture. The vendor of the PDMS pre-polymers offers two pre-polymer fluids, the base and the curing agent. The mixing ratio of the base and reagent is 10:1. If the base in the mixture ratio is increased, the final PDMS polymer would be softer. On the other hand, increasing the percentage of the curing agent would make the solidified PDMS harder. Therefore the exact ratio of the base and curing agent can be adjusted as needed to obtain the desired physical properties of the final PDMS polymers for different applications.
2) Mix the base and curing agent. The mixture of the base and curing agent needs to be stirred for several minutes to ensure complete mixing. The mixture can then be poured on the SU-8 master mold to form a particular thickness of PDMS layer. The thickness can be controlled by manipulating the weight of PDMS.

3) Degas PDMS mixture. The wafer is then put in a vacuum chamber for 15 minutes to get rid of the air bubbles.

4) Cure the PDMS. The curing process is normally done by heating the PDMS mixture to 65°C for 3 hours or 100°C for 30 min. The higher temperature and longer curing time can increase the stiffness of the polymer.

5) Demold the replica. Peeling the PDMS off the master mold needs to be done carefully because of the vulnerable microstructures on it. Fortunately, the excellent flexibility of PDMS makes it very rare to have these structures damaged. This flexibility also makes it possible to replicate some very complicated structures and provides us the opportunity to be more creative in our design.

6) Bond with the substrate. After the replica is prepared, certain holes can be drilled for the connection with tubes to introduce the fluidic samples or reagents. The PDMS itself can serve as the material for the structure microfluidic system. It can be bonded to glass or silicon substrate directly for reversible bonding. To prevent leakage at high pressure, the replica and substrate can be treated with plasma for surface modification and achieve irreversible bonding. Figure 1.6 shows a typical fabrication process to obtain a PDMS microfluidic chip.
1.4 Scope of the research

The research work presented in the dissertation aims to develop the essential components for centrifugal microfluidics such as valves, pump and mixer. In chapter 2, a magnetically actuated valving system is designed. The novelty is to use a flyball governor to achieve the successful control of the magnetic force, which is very compatible with centrifugal fluidic technologies. The major advantage of this new valving technology is that its burst frequency can be conveniently manipulated by adjusting the parameters of the mechanical system without changes in the fluidic pattern. Analytical analysis of the actuation mechanism has been conducted and the results have been presented and compared with the experimental ones. Our study has confirmed that the mathematical analysis can be used to predict the burst frequency of the valve. The actuators of the valve are isolated from the fluid system to prevent sample cross-contaminations.

In chapter 3, a novel pinch valve systems was designed and tested. The first one is based on the magnetic force and the second one is on the basis of spring force and the last one is a membrane valve. All valving system demonstrate good control of the fluid movement. The latter two valves are capable of sequential control. It proves that the flyball governor system is very compatible with centrifugal fluidic technologies. The major advantage of this new actuation technology is that its burst frequency can be conveniently manipulated by adjusting the parameters of the mechanical system without changes in the fluidic pattern.
Then as a proof of concept, a sequential valving system capable of metering and centrifugal sediment was developed in chapter 4 for plasma extraction from whole blood. The resulting residual cell concentration was less than 0.5%.

Next, in chapter 5, two types of inward pumping systems were designed and tested. The inward pumping is achieved in two steps. The first step is to close the channel by the valves demonstrated in chapters 3 and 4 to create an air-tight condition. Next a compression chamber is compressed with the help of the flyball governor system. The result shows that the two inward pump were capable of the pumping over a radial distance of 21mm in short time and achieve a pump efficiency of 100%. It thus improves the usage of space on the disc and paves the way to interconnect several functional units.

In chapter 6, a micromixer was developed based on the similar principle. The mixing chamber can be periodically compressed while altering the spinning speed. The results show that the flyball governor system can effectively agitate the chaotic mixing of the sample liquids by periodically deflecting the PDMS membrane of the mixing chamber. The mixing effect can thus be enhanced.
CHAPTER 2. A NOVEL MAGNETICALLY ACTUATED VALVE FOR CENTRIFUGAL MICROFLUIDIC APPLICATIONS

2.1 Introduction

In recent years, centrifugal microfluidic systems have gained popularity due to their intrinsic advantages in pulse-free pumping, complex integration and automation[4]. As an essential component for fluid control, valving has been extensively investigated in the microfluidic field. Various valves have been particularly proposed for centrifugal microfluidic devices. Among them, there are two widely used valves: the hydrophobic valve and capillary valve. The hydrophobic valve can be achieved by either hydrophobic patches [3] or geometrical changes [8] in the channel. The liquid can be eventually forced to pass the valve by increasing the rotational speed beyond the burst frequency. The capillary valve is based on the balance between centrifugal pressure and surface tension in a hydrophilic material[9]. All these valves as well as the siphon valve [10] are limited to the surface tension of the liquid especially for the highly wetting liquids[11]. To overcome the limitations of these passive valves, some sacrificial valves have also been reported such as wax valve[5], film valve[6] and dissolvable membrane valve[7]. They take advantage of the phase transition and can operate independently of the rotational speed or valving locations. However, the phase-change substance needs to be deposited in the microchannels, which adds complexity to the manufacturing process and the direct contact between the substance and the solution may cause cross-contamination. Additionally, these valves usually require external energy source for actuation. For instance, the wave valve needs to be actuated by laser heating. Therefore, there is a continuous demand to develop a reliable valving system for the centrifugal microfluidic platforms.

On the other hand, magnetic actuation has been extensively employed in a variety of microfluidic systems[12]. A main advantage of this mechanism is the manipulation of the fluid
without direct contact, which could be an important issue in biological, medical and chemical applications. Some researchers have extended its application to centrifugal microfluidics such as pumping\cite{13}, mixing\cite{14}, lysis\cite{15}, extraction \cite{16, 17} and separation\cite{18}. Although some earlier studies have demonstrated the implementation of magnetically controlled valve on PDMS microfluidic chip with good performance \cite{19, 20}, this mechanism has not been applied to the valving system for centrifugal microfluidics yet. One possible reason is that it is not difficult to seal the channel with magnetic force, but it becomes challenging to release the force to open the valve on a spinning platform.

In this chapter, we report a novel valve which adopted a flyball governor to convert the spin motion of the platform into the perpendicular movement of the magnet. The vertical movement of the magnet helps to control the magnetic force exerted on the fluidic channel. As a result, the valve’s open/close states can be controlled by the rotational speed of the fluidic platform fixed with the flyball governor.

2.2 The Design and Construction for the Prototype System

2.2.1 Design and Components

Figure 2.1 (a) shows the schematic diagram of an assembled centrifugal microfluidic platform. The close-in diagram of the valves above the assembled system shows working principle of the valves. When the permanent magnet is drawn to move downwards and press against the covering membrane, deflection of the membrane helps to close the flow channel. Figure 2.1 (b) presents an exploded view of the system showing all the major components and how they are assembled. The system consists of three main parts: a spin-stand, a disc stack, and a flyball governor. The disc stack includes a microfluidic disc (PMMA disc 2), magnet cylinders and the top and bottom magnet holders (PMMA disc 1 and 3) as shown in Figure 2.1(b).
The adoption of the flyball governor serves to provide an easy way to control the positions of these permanent magnets. It is very compatible with the centrifugal microfluidic platform because of the already available spinning power. The governor system is made up of sleeves, link arms, spring and flyballs. Two weights are hooked to the end of the arms as flyballs. It functions on the principle of spring pressure opposed by the centrifugal force. When the spindle rotates at a certain speed, the centrifugal force drags the flyballs outwards which in turn pulls the bottom magnet holder downwards through the linkage.

The microfluidic disc (disc 2) is sandwiched between disc 1 (holding the top magnets) and the disc 3 (holding the bottom magnets). All the PMMA discs are of 4-inch in diameter and 1/8 inch in thickness. In particular, both disc 1 and disc 3 contain several CNC-machined holes that are designed to load the permanent magnets. The top magnet holder (disc 1) and the microfluidic disc (disc 2) are fastened to the spindle with nuts, while the bottom magnet holder (disc 3) is designed to slide freely along the spindle. Disc 3 is attached to a sleeve that belongs to the underlying governor system. In this particular design, four valves will be designed on the platform, with each valve requires one pair of permanent magnets. A total of 8 cylindrical permanent magnets (size: ¼ inch diameter × ¼ inch thick; grade: N48; pull force: 6.1 pounds) are therefore used to form four facing pairs, four on each of the discs 1 and 3. The holes in the top holder are slightly larger than the diameter of the magnet cylinder to allow the magnet to freely press the PDMS layer against the substrate. The holders can also prevent the magnets from popping out during the rotation for safety. The bottom magnets are fixed inside the holes with glue so that they move together with disc 3. As shown in Figure 2.1(b), a 2mm thick washer is placed between the microfluidic disc and the top magnet holder to avoid direct contact between them, and intimate contact is enabled between the bottom magnet holder and the substrate to produce the optimized
magnetic force. The spacer is larger than the width of the fluid channel to ensure the channel can be completely closed during valve operation [18].

Figure 2.1(c) shows the design and positions of the three discs. In this specific design, four valves were incorporated in the microfluidic system. Each of them requires one pair of magnets. The magnets in disc 1 are free to move vertically, and the magnets in the bottom disc (disc 3) are fixed on the substrate. The disc 2 is fabricated by bonding a PDMS microfluidic system to a PMMA substrate. Figure 2.1(d) shows the schematic design of the microfluidic platform with four valves integrated on it. The flow channel of each valve connects two circular chambers, one as “loading chamber” and the other one as the “collecting chamber”. The attractive force between magnets is applied on the deformable PDMS membrane of the flow channel through a ball spacer.

2.2.2 Construction and Operational Principle

Figure 2.2 (a) shows a photo image of the flyball governor fixed on a PMMA disc (disc 3). Figure 2.2 (b) shows a photo image of the assembled prototype system. The spin-stand is equipped with a brushless motor with controller (BLDC-38S, Zhengke Motor Co, China) to spin the discs. The motor is installed on a metal frame with its output shaft connected to the spindle through a coupling. The spinning speed is monitored using a LED tachometer.

The operation of the valve is based on the interaction and balance of the magnetic force and the elastic force of the flexible covering membrane of the flow channel. When the flyball governor is stationary or rotates at low speed (600 rpm in this case) as shown in Figure 2.3 (a), the spring force dominates, and disc 3 stays at top position and the gap between disc 1 and disc 3 are minimum, magnetic force between the magnet pairs help to keep the valves closed as shown in Figure 2.3(b). The elastic force of the membrane and the fluid pressure produced by centrifugal force cannot overcome the magnetic force.
Figure 2.1 Schematic diagrams of the valves integrated in a centrifugal microfluidic platform. (a) Schematic diagram of the valve system consisting of three PMMA discs and a flyball governor. (b) The exploded view of the assembly of the system to illustrate the various components. (c) Exploded view showing the design and positions of three discs. (d) Top view of the assembled disc stack. The top and bottom magnets are aligned to the center of the channels.
Figure 2.2 Photo images of the fabricated system: (a) Image of the flyball governor; (b) Image of the complete system after the top magnet holder and the microfluidic disc are assembled.

Figure 2.3 Operational principle of the microvalve. (a) A side view of image of the valving system in the “closed” state with the spindle turning at a low rate (600rpm); (b) Schematic of the deformation of the channel when pressed by the magnet through a ball spacer; (c) The schematic of the fluid being blocked and stays in the loading chambers; (d) Side view image of the valving system in the “open” state with the spindle rotating at 1100rpm. (e) Schematic diagram showing the channel in “open” state when the magnetic force is removed. (f) Top view schematic diagram of the fluid being delivered to the collection chamber when the valve is open.
The flow channels connecting the “loading chambers” and the “collecting chambers” are therefore closed and the sample fluids stay in the “loading chambers” as shown in Figure 2.3(c). These valves are therefore classified as the “normally closed” valves. When the spin speed of the flyball governor reaches a critical value, the centrifugal force drives these balls outward and pulls disc 3 to slide downward along the shaft as shown by the photo image in Figure 2.3(d). As can be seen in the image, the bottom magnet holder (disc 3) is separated from the microfluidic disc (disc 2). The spring is compressed and the platform reaches a new equilibrium as the pulling force balanced by the spring force. As the distance between the top and bottom magnets increases, the magnetic force between the magnet pairs is significantly reduced. When the magnetic force is lower than the elastic force of the flow channels in valves, the flow channels connecting the loading and collecting chambers is open as shown schematically in Figure 2.3(f). Fluid in the loading chamber flows to the collecting chamber under the influence of centrifugal force as shown in Figure 2.3(g). When the spin speed of the flyball governor drops below a specific rate, the centrifugal force drops and the spring force helps to push disc 3 upward again. The distance between the top and bottom magnets is reduced and the magnetic force increases. The magnetic force overcomes the elastic force of the PDMS membrane and the flow channels are closed again.

2.2.3 Dynamic Analysis

A force analysis was performed to calculate the burst frequency of the valve. The geometric demonstration of the linkage of the flyball governor is shown in Figure 2.4(a). For the reason of simplicity, the radius of the sleeves is neglected. The linkage rotates around the shaft axis through points A and C. The length of link CD is \( l \) and the length of the link AB is \( L = 2l \). Point C is right in the middle of link AB. \( \theta \) denotes the angle between the axis and link AB. Free-body diagrams for the linkage and the bottom holder are illustrated in Figs. 2.4(b) and 2.4(c). The
centrifugal forces exerted on links AB and CD and the flyball are denoted as $F_1$, $F_2$, and $F_3$, respectively. The magnitudes of these forces are as follows,

\[ F_1 = \frac{1}{2} m_{CD} \omega^2 l \sin \theta, \quad (2.1) \]
\[ F_2 = m_{AB} \omega^2 l \sin \theta, \quad (2.2) \]
\[ F_3 = 2 m_B \omega^2 l \sin \theta, \quad (2.3) \]

where $m_{AB}$, $m_{CD}$ and $m_B$ are the masses of the links AB, CD, and CD respectively.

The moments about point C in the free-body diagram for the linkage are calculated as,

\[ \sum M_C = 0, \quad F_1 \frac{1}{2} l \cos \theta + F_2 l \cos \theta = F_{Ax} 2 l \cos \theta. \quad (2.4) \]

Similarly, the moments about point D in the free-body diagram for the link AB are,

\[ \sum M_D = 0, \quad F_3 l \cos \theta + F_{Ax} l \cos \theta = F_{Ay} l \sin \theta. \quad (2.5) \]

The pulling force $F_{Ay}$ is obtained from Eqs. (1)-(5) as,

\[ F_{Ay} = \left( \frac{1}{4} F_1 + \frac{1}{2} F_2 + F_3 \right) \tan^{-1} \theta = \left( 2 m_B + \frac{1}{2} m_{AB} + \frac{1}{8} m_{CD} \right) \omega^2 \cos \theta. \quad (2.6) \]

In order to pull the bottom holder (disc 3) downward, the pulling force must exceed the sum of the maximum magnetic force $F_{m0}$ and the preload of the spring $F_{s0}$ (see Figure 2.4(c)),

\[ 2F_{Ay} \geq F_{m0} + F_{s0}. \quad (2.7) \]
\( F_{s0} \) can be tuned with the adjusting nut and written as

\[
F_{s0} = 2kl(\cos \theta_0 - \cos \theta_1),
\]  

(2.8)

where \( k \) is the spring stiffness, \( \theta_0 \) is the angle when the spring is at equilibrium state, and \( \theta_1 \) is the angle when the spring is pre-tensioned by the nut. Submitting Eqs. (2.6) and (2.8) into Eq. (2.7), the burst angular frequency \( \omega_0 \) can be obtained as,

\[
\omega_0 = \sqrt{\frac{F_m + 2kl(\cos \theta_0 - \cos \theta_1)}{(4m_B + m_{AB} + \frac{1}{4}m_{CD})l \cos \theta}}
\]  

(2.9)

Eq. (2.9) provides the guidelines to adjust the burst frequency of the valve by manipulating the configurations of the system. For instance, if a higher burst frequency is in need, a series of design options can be considered, such as using magnets with higher strength, decreasing the thickness of the PDMS layer or substrate, reducing weights of the flyballs, and increasing the preload of the spring. It also suggests that the burst frequency of the valve can be altered without any change in the design of the microfluidic device as long as the magnetic force is strong enough to close the valve in the beginning.

The initial distance between the two sets of magnets is about 10mm (thickness of the PMMA disc and the PMDS layer). The magnitude of \( F_{m0} \) can be estimated theoretically [21] and experimentally. It is should be noted that the spring is further compressed as the bottom magnet holder (disc 3) moves downwards. The gap between the microfluidic disc (disc 2) and the bottom magnet holder (disc 3) \( \Delta x \) is kept zero before the separation. After the separation occurs (see Figure 2.3 (d)), \( \Delta x \) can be expressed as,

\[
\Delta x = 2l(\cos \theta_1 - \cos \theta) = 2l(\cos \theta_1 - \frac{2F_m + 2kl \cos \theta_0}{(4m_B + m_{AB} + \frac{1}{4}m_{CD})l \omega^2 + 2kl}) \text{ when } \omega > \omega_0
\]  

(2.10)
It is clear that the gap increase with the rationing speed. Additionally, the magnetic force $F_m$ is coupled with $\Delta x$, so it is difficult to obtain an analytical expression for $\Delta x$. However, since the magnitude of the magnetic force decreases quickly [19] with $\Delta x$, it can be neglected in the following calculation.

### 2.3 Fabrication of the Microfluidic Disc

The master mold of the fluidic system was first fabricated using UV lithography of SU8, a negative tone photoresist. The PDMS layer was then created by casting silicon elastomer (Sylgard184, Dow Corning) on the master mold. The fabrication flowchart is schematically demonstrated in Figure 2.5.

![Figure 2.5 Schematic procedure to fabricate the microfluidic disc](image)

In fabrication of the master mold, a 200 µm thick layer of SU-8 100 photoresist (MicroChem, USA) was first spin-coated on a 4-inch silicon wafer. The wafer was then soft baked at 75°C for 15min and 95°C for 2h on a hotplate. Next, patterns on a mask were transferred to the photoresist by UV exposure (700mJ/cm²). After post baking at 75°C for 10min and 95°C for 40 min, the wafer was immersed in the developer to remove the unexposed area to obtain the master mold of cured SU-8 polymer. In the cast molding process, 8g PDMS was mixed in a 10:1 (m/m) of base/curing agent ratio and casted on the master mold. The sample was first placed in a vacuum...
desiccator for 15min for degassing, and was then cured at 85°C in oven for 1hr. The cured PDMS layer was subsequently peeled off the mold and the holes were punched using sharpened blunt needle. The PDMS layer was then cut into 9cm diameter disc with a thickness of 4mm. Next, the PDMS replica was bonded on a 1/8 inch thick and 4-inch diameter PMMA substrate. To bond the PDMS layer to the PMMA disc, a thin layer of PDMS was first spin-coated on it at 1000rpm for 30s and cured at 85°C in oven for 20min. The surfaces of the PDMS replica and substrate were treated with oxygen plasma (50W for 30s in a Bransen plasma asher) and then bonded together. Finally, an 800μm diameter aluminum ball (Mcmaster, USA) was carefully positioned on top of each micro channel under microscope with glue. The sphere works as a spacer to ensure the channel can be fully closed when pressed by the magnet [18]. Figure 2.6 shows a close-in image of the assembled microfluidic disc. Each of the prototype valving units in the PDMS layer features two chambers and a micro channel. Filleted corners were adopted to minimize the influence of the capillary force. The diameter of the two chambers is 8mm. The flow channel has a cross-section of 100μm × 200μm and a length of 10mm. The magnets and the ball spacer were all aligned to the center of the channel. To obtain a better view of the ball spacer, the magnet above it was removed as shown in Figure 2.6(b). The fluid is generally introduced into the loading chamber before the tests. When the disc is spun at a speed above the bursting rate for valve, the flow channel connecting the two chambers is un-blocked (valve opened), the liquid is propelled by the centrifugal force to move into the collecting chamber.
Figure 2.6 Photo images of a prototype valve with the magnet and the ball spacer installed and aligned above the flow channel of the valve. (a) Top view of the valve with top magnet. (b) Top view of the valve with the magnet removed to show the ball spacer for better observation.

### 2.4 Experimental Results and Discussions

To verify the functionality of this new design of pinch valves actuated by a pair of magnets and a flyball governor, a microfluidic disc containing four pinch valves was designed, fabricated, and tested. The flow channels of the valves are evenly distributed in the circumferential direction of the disc. A camera was positioned above the platform to capture the still image of the fluid samples. We first examined the performance of the flyball governor without any sample loading. The system were spun at various rotating speeds. The distances that the magnetic holder (disc 3) traveled $\Delta x$ were recorded and compared with the analytical results from Eq.(2.10) in Figure 2.7. They demonstrate the similar trend. The significant separation was observed at around 800rpm. Then the gap gradually increases with the rotating speed when the spring is continuously compressed. As the rotating speed reaches 1300rpm, the spring is compressed to the limit of the compression.
Next the burst frequency was then measured while the spinning speed of the flyball governor was adjusted. The initial spinning speed was set at 300 rpm. The spinning speed was increased in steps of 50rpm. The disc was then centrifuged for 30s and stopped to check if any fluid passed the valve. If fluid samples were observed to have moved to the collecting chambers, the corresponding spinning speed could be regarded as the burst frequency of the valve. Figure 2.8 demonstrates a series of images showing a valve in operation. A volume of 50 μl red food dye was injected into the loading chamber first as shown in Figure 2.8 (a). Figure 2.8 (b) shows an image when the spinning speed was increased to 600rpm, in which the top magnet was removed for observation purpose. As can be seen from the image, a segment of liquid had been transported to the flow channel followed by some air but blocked by the ball spacer. The valve remained closed. Next, when the spinning speed reached 800rpm, all the liquid in the “loading chamber” was delivered to the “collecting chamber” as shown in Figure 2.8 (d). The burst frequency of the prototype valve in this case can be estimated to be around 800rpm. Next, a series of experiments were performed to investigate the factors affecting the burst frequency of the valve: the mass of the flyball, the preload of the spring, and the magnetic strength of the permanent magnets. The experimental results are compared with the theoretical values as predicted using Eq. (2.9).
Figure 2.8 Experimental images for the sample liquid passing through a prototype valve: (a) The sample liquid loaded in the chamber; (b) The disc was spun at 600rpm for 30s; (c) Close-in image with the top magnet removed for better view. The flow was stopped at the ball space indicating the valve was still closed; (d) Sample fluid was delivered to the collection chamber at 800rpm.

The experimental results are presented in Figure 2.9 together with the theoretical values predicted using Eq. (2.9). As can be seen from these results, the theoretical and experimental results demonstrated good agreement. The measured burst frequency ranged from 800rpm to 1600rpm. The measured burst frequencies are lower than the theoretically predicted ones. The vibration of the system during the rotation might have contributed to the reduction in the burst frequencies because it might have affected the contact between the top magnet and the PDMS layer. From the experimental results, it can also be observed that the burst frequency of the valve can be easily tuned by adjusting the mechanical parts without any change in the fluidic design. In addition, the burst frequency is not sensitive to the valving location. Therefore, the design of this valving system provides great flexibility in the design of the fluidic system. Moreover, the actuators of the valve, including the magnetic holder and the flyball governor, are all isolated from the fluidic system. The microfluidic disc can therefore be made disposable, helps to avoid any possible cross-contamination.
Figure 2.9 Comparison of experimental results of burst frequencies of the prototype valves with theoretical predictions under various settings: (a) with varying flyball mass; (b) with varying spring preload. The stiffness of the spring is 1.47 N/mm. The length of the arm is 8.9 cm. The initial angle between the arm and the spindle is 37°.

The response time of the valve in the experiment is around 1~3s depending the acceleration and deceleration of the motor and no leakage was observed during the experiment.

The novelty of this work is that we proposed a mechanical system to apply the concept of pinch valve on a rotating disc successfully for the first time. The capability to control burst frequency can add flexibility to the design of the fluidic system. For example, the centrifugal separation is a typical procedure in biomedical analysis. When we apply it on the disc, the sample should first be confined in a sedimentation chamber with a closed valve. After the centrifugal
separation is completed, the valve should be opened to transfer the sample to other chambers for further analysis. As a result, burst frequency of this valve must be set to be between the separation frequency and the limit frequency at which the cell might break apart. So the burst frequency of the valve may need to be adjusted according to the properties of different cells. In this case, our proposed valving system can effectively solve this problem without changing the design of the fluidic system and enable a more general platform for multiple applications. Though this new type of valves have many advantages for applications in centrifugal microfluidic systems. It also has some limitations. First, the number of valves that can be integrated into a single disc is limited because of the physical sizes of the magnets and the potential interaction between neighbouring magnets. Secondly, this valve itself is not suitable for sequential or individual control because the magnetic forces for different pairs of magnets are exerted or removed simultaneously when the flyball governor moves upward or downward. Nevertheless, this valve can be easily incorporated with other types of valves to achieve sequential control owing to its simple structure and variable burst frequency. Hence, it is still a useful alternative to the existing valving technologies.

2.5 Conclusion

This study has confirmed the feasibility of this new type of valving system. The main novelty of this work is to use a flyball governor to achieve the successful control of the magnetic force, which is very compatible with centrifugal fluidic technologies. The major advantage of this new valving technology is that its burst frequency can be conveniently manipulated by adjusting the parameters of the mechanical system without changes in the fluidic pattern. Analytical analysis of the actuation mechanism has been conducted and the results have been presented and compared with the experimental ones. Our study has confirmed that the mathematical analysis can be used to predict the burst frequency of the valve. The actuators of the valve are isolated from the fluid
system to prevent sample cross-contaminations. The simple fabrication process also makes it suitable for disposable applications without external power source. It is expected that the application of this actuation mechanism can be extended to other potential fluid handling for the centrifugal microfluidics such as sample loading, mixing and pumping. Additionally, this design enables to switch the magnetic field for the centrifugal platform during operation. It can also be used in the related fields such as ferroliquid microfluidics and magnetic bead-based immunoassay
CHAPTER 3. A PINCH VALVE FOR PDMS BASED CENTRIFUGAL MICROFLUIDIC PLATFORMS BY USING SPRING PLUNGER

3.1 Introduction

In the previous chapter, a magnet actuated pinch valve is successfully integrated into the centrifugal microfluidic system. Though it functioned well, there exist two drawbacks. First, the size of the valve is still large for a single disc and the potential interaction between neighbouring magnets. Secondly, the valve is not suitable for sequential or individual control because the magnetic forces for different pairs of magnets are exerted or removed simultaneously. To overcome these limitations, a spring plunger was used to replace the magnet to create a new pinch valve as reported in this paper. Sufficient pressure can be generated by a spring plunger to pre-stress the PDMS channel to an initially closed state. The flyball governor drives the PDMS layer to release the compression when it spins at a particular speed. Therefore, the “on”/“off” control of the flow is simply achieved by controlling the rotating speed. More importantly, the height of the spring plungers can be adjusted. Therefore, the valves can be opened at different burst frequencies to achieve sequential control, which is essential for many biological and chemical applications.

3.2 Materials and methods

3.2.1 Design of the pinch valving system

Figure 3.1(a) shows the schematic design diagram of an assembled centrifugal platform with a pinch valve. There are three major parts of the system: the flyball governor, the actuation disc with the spring plunger fixed on it, and the microfluidic disc. The microfluidic disc is fixed on top of the shaft, only rotates with the shaft without vertical sliding. The bottom part of the flyball governor is fixed on the shaft and rotates with it. The actuation disc is fixed on the top part of the flyball governor and rotates with the shaft, and at the same time, can slide along the shaft as
the centrifugal force of the flyballs pulls it downward. The supporting spring between the top and bottom parts of the flyball governor provides the restoration force to balance with the centrifugal force. The micro-sized spring plungers are fixed on the actuation disc. When the system is in stationary state, the supporting spring pushes the actuation disc up and the spring plungers applied pressure to the microfluidic disc. As the system rotates, the centrifugal force helps to overcome the force of supporting spring in flyball governor, and pulls down actuation disc and take the spring plungers to move downward.

The actuation of this valving system is based on the interaction between the elastic forces of the membrane covering the fluidic channel and the spring plunger. At initial state, the spring plunger is pressed against covering membrane of the flow channel. The deflection of the membrane helps to close the flow channel and to maintain the channel in “closed-state”. As the system starts to spin, the flyball governor is activated by centrifugal force to pull the spring plunger away from the channel and eventually “open” flow channel, and the valve is in “open-state”. The platform consists of three parts from the top to bottom: a microfluidic disc, actuator disc, and the flyball governor. The spring plungers are embedded in the actuator disc. They are used to apply pre-set forces to the flow channels, and cause the covering membrane of the flow channels to deflect to block the flow. Therefore, these valves are initially at the “normally closed” states. The actuation disc was connected to the flyball governor system with screws. In this way, the actuation disc can be easily replaced for different purposes. As the platform spins, the centrifugal force exerted on the flyballs compresses the supporting spring through the linkage, and in turn pulls down the actuation disc to release the pressure forces applied by the spring plungers on the membrane. At low spinning speed, the tip of the spring plunger can still maintain a strong pressure force to close the channel. However, when the spinning speed reaches a critical value (“burst frequency”), the
valve is opened to allow the flow to pass through (see Figure 3.1 (b)). The working principle of the spring plunger is shown in Figure 3.2. The spring plungers (McMaster, USA) were inserted into the actuator disc. The body diameter is 1/4 inch. The nose is 1/4 inch long and the tip is 1/8 inch in diameter. They can provide a maximum nose force as 4N when fully compressed.

Figure 3.3 shows three photo images of the assembled valving system during operation. The system is equipped with a brushless motor to spin the discs through the coupling. The flyball governor comes with three flyballs. Screws and spacers were used a flyball in our experiments so that the weight can be adjusted conveniently. A metal tube is placed to surround the shaft so that the actuation disc can move downwards to press the supporting spring. The stiffness of the supporting spring is 1.47N/mm. The microfluidic disc and the actuation disc have the same size of 4 inch in diameter. The microfluidic disc was fabricated by bonding the PDMS layer with a PMMA substrate. The actuation disc was fabricated by 3D printing using ABS as structural material to reduce the weight of the system.
Figure 3.2 Demonstration of the spring plunger when it is (a) at rest and (b) compressed

Figure 3.3 Photo images of the valving system: (a) Side view image of the fully assembled valving system in close. (b) Close-up view of the valving position. (c) Side view image of the valving system when the flyball governor is drawn downwards to open the valves at 1200rpm.

3.2.2 Fabrication of the microfluidic disc

Figure 3.4 gives the flow chart for the fabrication process of the microfluidics disc. The master mould of the fluidic system was first obtained by the conventional UV lithography of 500 μm thick SU-8 layer on silicon wafer. The detailed fabrication process is similar to the one described in the previous chapter. The difference is that during the operation, this microfluidic disc is placed in a face-down fashion. The inlet of the loading chamber is drilled on the PMMA disc instead of the PDMS layer. As a result, the sample can be easily introduced into the system after the disc is mounted. The PDMS structure was fabricated by casting silicon elastomer (Sylgard184,
Dow Corning, USA). For all the microfluidic devices, we prepared the PDMS by a 10:1 of base/curing agent ration and cured the PDMS at 85°C for one hour. To ensure the channel can be fully closed, an 800μm diameter aluminium ball (McMaster, USA) was carefully placed on top the microchannel under the microscope with glue. The location of the sphere ball is designed as the valving position. Next, another PDMS layer was casted to immerse the sphere ball. Then the venting holes and center hole were punched into the replica. To prepare the substrate for the PDMS replica, a series of holes (inlets and center hole for the shaft) were first drilled in the PMMA disc and a thin layer of PDMS was spin-coated on it. Finally, the surfaces of the PDMS replica and PMMA substrate were treated with oxygen plasma (50W for 30s in the Bransen Plasma Asher) and bonded together with alignment. As a result, as the covering membrane of the channel was compressed by the spring plunger, the PDMS membrane deformed to block the flow channel, and serves as a functional valve.

3.3 Improvement on the experimental Setup

Figure 3.6 shows the schematic diagram of the proposed Lab-on-CD instrumentation technology. For a better control of the mechanical system, several improvement was made on the basis of the previous system used for the magnetically actuated valve. First, a three-flyball governor was used instead of two-flyball governor so as to increase the stability of the whole system during operation. Second some parts including the actuation disc was fabricated by 3D printing to minimize the weight of the rotary system as shown in Figure 3.7. Besides, the position of the spring plungers can be precisely defined in order to get a better alignment with the microfluidic channel i.e. the valving position. A mini wireless camera (2cm× 2cm×2cm) is fixed on the top of the system, and rotates synchronously with the disc to observe and record the movements of the liquids in the microfluidic disc.
Figure 3.4 Fabrication procedure for the pinch valves

Figure 3.5 Photo image of a microfluidic disc with three valving units
In addition, to precisely control the spinning speed, a control system was set up for the motor. The structure is shown in Figure 3.8. A brushless DC motor (BLDC-38S, Zhengke Motor Co, China) is used to spin the platform. BLDC-38S has been internally integrated with driver and Pulse Width Modulation (PWM) speed control modules. The speed of the motor is originally designed to be adjustable through a potentiometer. In order to realize the programmable control of the motor speed, the external wires have been reconfigured, as shown in Figure 3.8. The potential of the green terminal will be controlled by the programmable device rather than by a potentiometer.
A Hall sensor is used to measure the RPM of the motor and feedback to the control unit. The control system is constructed based on the USB-6008 (National Instruments, Austin, TX) data acquisition (DAQ) devices, which interacts with the computer to acquire the data from the Hall sensor and adjust the Pulse Width Modulation (PWM) module in the motor so as to realize the control of the motor speed in a closed loop. The rotating direction can be changed through a switch connected to the GND terminal and the direction control terminal (gray) of the motor. If the switch is closed, the motor will rotate in clockwise, otherwise, in counter-clockwise.

The control system is implemented using LabVIEW (National Instruments, Austin, TX) which is able to interact with physical devices like actuators and sensors through the DAQ device. In order to stabilize the governor system around a desired distance with the Lab-on-CD disc, a Proportional Integral Derivative (PID) Controller is used to control the spinning speed of the motor. The block diagram of the LabVIEW program is shown in Figure 3.9.

![Figure 3.8 The motor Control System](image)
3.4 Results and discussions

3.4.1 Single valve operation

In order to demonstrate the functionality of the pinch valve, three prototypes of pinch valve were prepared on the same disc with the operational principle shown schematically in Figure 3.10. Each of them features a loading chamber, a collecting chamber and they are connected by a microchannel. They are all 500 μm in depth. Both the chambers are 6mm in diameter. The channel is 300 μm wide. Filleted corners were utilized to minimize the impact of capillary force. The ball spacer was aligned to the center of the spring plunger. A volume of 10μL red food dye was injected into the loading chamber. The wireless camera positioned above offers the real-time image for observation. We first tested the microfluidic design with no pinch valve. The microfluidic design then can be regarded as a hydrophobic valve due to the capillary force. It is found that the liquid flowed to the collecting chamber at speeds of ~600rpm. Next the performance of microfluidic design equipped with the valves were investigated. Figure 3.11 illustrates the schematic side-view and top-view diagrams and experiment images of the valves in operation. As the spinning speed
reached 910rpm, the liquid was delivered to the collecting chamber. Thus, this value can be regarded as the burst frequency in this case.

![Diagram of disc design](image1)

**Figure 3.10** (a) Schematic of the disc design to conduct the single valving system (b) Side view of the arrangement of the microfluidic disc and the actuation disc

![Diagram of demonstration](image2)

**Figure 3.11** Single valving demonstration. (a1-a3) Demonstration of the valve at low spinning speed. The spring plungers pins the valve to close. (b1-b3) Demonstration of the valve valving system as the spinning speed increases. The spring plungers moved down to open the valve. (a3) and (b3) Video snapshots showing the transition from “closed” to “opened” valve positions.

### 3.4.2 Dynamic analysis of the single valve system

For a better understanding of valving system, a force analysis is conducted to investigate the burst frequency. Figure 3.12(a) shows the schematic of the simplified valving system. $k_1$ and $k_2$
are the stiffness of the spring plunger and the supporting spring, respectively. Here we only considered the stiffness of the spring plungers and the supporting spring and neglect the mass of the actuation disc, the linkage and the flyballs. Initially, the three spring plungers and the supporting spring are compressed as shown in Figure 3.12(b). $F_0$ denotes the preload of the supporting spring which is balanced by the total spring force from the three plungers. $L$ is the free length of the supporting spring. $L_1$ is the length of the supporting spring after assembly. Therefore, $L_1 = L - \frac{F_0}{k_2}$.

And in our experiment $L=52$mm. As the system starts to spin, the centrifugal force is exerted on the flyball (Figure 3.12(c)) and continues to further compress the supporting spring and release spring plungers. Here we assume that when the spring plunger is fully relaxed, the valve starts to open and the corresponding spinning speed can be regarded as the burst frequency. So we can have

$$L_2 = L - \frac{F_0}{k_2} - \frac{F_0}{3k_1} \quad (3.1)$$

$$F_{Ay} = \frac{2F_0}{3} \quad (3.2)$$

$$F_{cent} = m_b(a + 2l\cos\theta)\omega_0^2 \quad (3.3)$$

Where $\theta$ denotes the angle between the axis and link AB; $F_{cent}$ is the centrifugal force and $m_b$ is the mass of the flyball governor; $F_{Ay}$ is the pulling force at joint A; $a$ is the distance between the beam to the axis ($a=15$mm); $2l$ is the length of the beam ($l=15$mm); $\omega_0$ is the angular velocity for the burst frequency.
Figure 3.12 Schematic of the simplified valving system and (b) the free-body diagram of the linkage after assembly and (c) when the spring plunger is fully relaxed to open the valve.

The moments about point C and D in the free-body diagram for the linkage are calculated as,

\[
\sum M_C = 0, \quad F_{cen}(L_2 - 2l \cos \theta) = F_{Ax} L_2 \tag{3.4}
\]

\[
\sum M_D = 0, \quad F_{cen} l \cos \theta + F_{Ax} l \cos \theta = F_{Ay} l \sin \theta \tag{3.5}
\]

Besides,

\[
\cos \theta = \frac{l_2^2 + l^2 - (2l)^2}{2l_2^2} \tag{3.6}
\]

Thus, the burst angular frequency \( \omega_0 \) can be obtained from Eqs(3.1)-(3.6) as,

\[
\omega_0 = \sqrt{\frac{\frac{2}{3} \tan \theta F_0 (L - \frac{F_0}{k_2})}{m_b (2l \sin \theta + a) \left\{ 2 \left( L - \frac{F_0}{k_2} - \frac{F_0}{k_1} \right) - 2l \cos \theta \right\}^2}} \tag{3.7}
\]

where \( \theta = \cos^{-1} \left( \frac{(L - \frac{F_0}{k_2} - \frac{F_0}{k_1})^2 - 3l^2}{2l (L - \frac{F_0}{k_2} - \frac{F_0}{k_1})} \right) \)

It is clear that the burst frequency relies on the parameters of the mechanic system. Next, a series of experiments were conducted to investigate the effects of the flyball mass and the preload of the supporting spring on the burst frequency of a single valve. The results are shown in Figure 3.13. It is apparent that lighter flyball and greater preload of the supporting spring can lead to higher burst frequency. The burst frequency can range from 820 RPM to 1580 RPM. The results
have proved that this valving method offers great flexibility in the design of the centrifugal microfluidic devices. Moreover, the theoretical and experimental results demonstrate the same tendencies. The theoretical result can provide a guidelines for the design of the valving system. The difference is mainly caused by the simplification of the system and the neglect of the capillary pressure in the channel.

![Figure 3.13](image) Measured burst frequencies of the valve vs the theoretical result as the function of the flyball mass (a) and the preload of the supporting spring (b) respectively.

### 3.4.3 Sequential control of multiple valves

In applications requiring complex fluidic control, it is often desired that multiple valves can be used to achieve different stages of the flow. The pinch-valve reported in this paper can be readily adopted in sequential control of multiple valves for such applications. For demonstration purpose, a microfluidic disc was designed and fabricated using two cascaded valves as schematically shown in Figure 3.14. An intermediate chamber was added between the loading chamber and the collecting chamber for demonstration purpose. Two pinch valves were fabricated on the microfluidic disc. The corresponding spring plungers were placed at different height with the height difference d=4mm to generate different displacements as shown in Figure 3.15(c1). In stationary state or at low spinning speed, both valves are in “closed” state as shown in Figure
As the spinning speed increased to 1600 rpm, valve 1 opened first and the liquid flowed into the intermediate chamber while valve 2 remained closed as shown in Figure 3.15(b1-b3). When the spinning speed reached 2200 rpm, valve 2 opened up and the liquid sample flowed into the collecting chamber as shown in Figure 3.15(c1-c3). Particularly, Figure 3.15 (a3), (b3) and (c3) are the corresponding video snapshots.

The gap between the actuation disc and the microfluidic disc when the system is in stationary state or at lower spinning speed can be manipulated to adjusted the preload of the supporting spring. The preload of the supporting spring then determines the burst frequencies required for opening of the valves. Tests were conducted to establish the relationship between the burst frequencies of the valves and the preload of the support spring of the flyball governor. Similar dynamic analysis was conducted. The results are shown in Figure 3.16. It can be seen from the results that the burst frequencies increase as the preload of the supporting spring of the flyball governor.

3.5 Conclusion

In conclusion, a new valving technology combining the conventional pin valve and flyball governor is successfully designed, constructed, and tested for application in centrifugal microfluidic platforms. In addition to the intrinsic advantages of the pin-type valves, its burst frequency can be easily manipulated by adjusting the parameters of the mechanical system without changes in the fluidic pattern. This type of valves comes with many advantages such as simple structure, robust operation, low dead volume and leakage, and non-contact with the sample. In addition, this new valve is capable of multiple valving operation and bears high potential for comprehensive assay integration, automation and parallelization on the disc. Sequential control of multiple valves can be easily realized.
Figure 3.14 Schematic of (a) the disc design to conduct the sequential valves and (b) the actuation disc with two spring plungers arranged at different height d=4mm.

Figure 3.15 Sequential valving demonstration. (a1-a3) Demonstration of the single valving system at low spinning speed. Both the two valves are closed. (b1-b3) Demonstration of the sequential valving system at intermediate spinning speed. The spring plungers are drawn away to open valve 1 while valve 2 still closed. (c1-c3) Demonstration of the sequential valving system at high spinning speed. The spring plungers are further drawn away to open both valve 1 and valve 2.
Figure 3.16 Measured burst frequency of the valves vs the theoretical result varies with preload of the supporting spring.
CHAPTER 4. A PINCH VALVE BASED METERING AND EXTRACTION OF PLASMA FROM WHOLE BLOOD ON THE CENTRIFUGAL MICROFLUIDIC SYSTEM

4.1 Introduction

In the past several decades, an important trend has emerged in the analytical chemical and biomedical fields, that is, the development of miniaturized measurement instruments. These devices aim to integrate multiple analysis steps into one single, automated chip. With this regard, microfluidic systems have gained much attention to achieve this goal. These systems usually consist of a set of microfabricated chambers and channels, combined with a method of microfluidic pumping and valving for fluidic movement. As a result of this miniaturization, the microfluidic system features several important advantages such as low consumption of the sample and reagent, short analysis time, low cost and automatic operation. The high potential of these systems already gives birth to a considerable number of commercially available diagnostic devices.

The plasma extraction from the whole blood is the foundation for many medical diagnostics. The performance of the extraction process is usually determined by the residual cell concentration and the operating time. Besides, the extraction process should enable the integration with subsequent operations directly to avoid the troublesome interconnection or any possible contamination. Conventional sized plasma extraction performed in the hospital requires large-scale floor-based centrifuges which are bulky. The whole process is time-consuming and needs skilled laboratory technician. As a matter of fact, various microfluidic systems have been developed to achieve automatic blood separation and obtain a cell-free plasma sample. For example, dielectrophoresis was employed to separate the blood cell based on the dielectric properties of the cells[1]. This method is usually capable of diluted blood and its capability compromises when it comes to blood with high cell concentration. Besides, the process requires delicate operations. These devices has also been demonstrated based on microfilter[22, 23],
Zweifach–Fung Effect, bends in microchannel[24], cross-flow filtration[25] and acoustic waves[26].

As shown in Chapter3, we have already made significant progress in the development of the sequential control on a centrifugal microfluidic platform. As a proof of concept, we chose it as an example to demonstrate the potential biomedical application of the new pinch valve system.

As for the centrifugal microfluidic system, limited work has been demonstrated yet in spite of it potential as an ideal platform to accomplish the extraction of plasma from whole blood. The inherent centrifugal force can not only provide a pumping force to drive the sample but also enables centrifugal separation due to the density difference. In addition, the centrifugal microfluidics is also capable of multiplexing, automation and miniaturization. Some researchers have performed plasma extraction using centrifugal microfluidic systems [27, 28]. However, the valving system in their designs were relatively complex and required complicated fabrication process. Therefore, it is necessary to develop a robust and simple plasma extraction system on the centrifugal microfluidic platform.

With the proposed design, much smaller amount of blood is needed. Other function may also be incorporated at later stage for diagnosis detections. For instance, the targeted cells can be first marked using fluoresce dye, then optically detected. Because the microfluidic disc is replicated in low cost with transparent polymer, optical detection can be achieved relatively easily.

4.2 Materials and method
4.2.1 Operational principle of the sequential valving system

In order to perform the plasma extraction process, the blood should be first metered to define the volume of the sample blood to be analyzed. As described in Chapter 1, the metering process can be simply realized by an overflow channel. When the volume of the blood reaches the
capacity of the metering chamber, the extra blood can be directed to a waste chamber through the overflow channel. Then the blood will be delivered to a sedimentation chamber for centrifugal separation. After the separation process is completed, the plasma can be transferred to a collection chamber for further analysis. Therefore, a valving system capable of sequential control becomes essential.

In Chapter 3, a pinch valve has been developed based on spring plungers. More importantly, its capability for sequential control has been tested. It can be further arranged to achieve sequential control. Figure 4.1 shows its working principle. The left figures are presented in the side view while the right figures show the top view of the fluidic design. The new valving system is proposed based on the same mechanism as described earlier. The actuation is realized by a flyball governor system. The spring plunger of valve 1 is lower than that of valve 2. The sample liquid is introduced into the loading chamber at the beginning and both the valves are closed. Then as the rotating speed increases, the flyball governor system is activated and gradually draw the two spring plungers away from the PDMS layer. Due to the different height of the spring plungers, valve 1 will open first to let the sample fluid flow into an intermediate chamber and at higher rotating speed, valve 2 will start to open to deliver the fluid to the designation chamber.

4.2.2 Design of the extraction system

After the sequential valving system is completed, it can be integrated into the plasma extraction system. Figure 4.2 (a) illustrates the proposed fluidic design. The extraction system contains two valves that can be operated sequentially on the basis of the previous sequential valving method. As the spinning speed increases, valve 1 opens first. The burst frequency of valve 1 is lower than that of valve 2. Figure 4.2 (b) shows the side view of the actuation disc and the microfluidic disc. The corresponding spring plungers for the valves are placed at different levels.

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Figure 4.1 working principle of the proposed sequential valves on the centrifugal microfluidic platform

Figure 4.3 illustrates a schematic design for the microfluidic system which is capable of metering and centrifugal sedimentation. The system contains three valves (one capillary valve and two pinch valve) that can be operated sequentially on the basis of the aforementioned valving method. First the blood is loaded in the loading chamber. With valve1 closed the blood is transferred to the metering chamber to define the volume of the sample needed to be analyzed. Then open valve1 and the blood is introduced to the sedimentation chamber while valve2 remains closed. Next, the red blood cells are sediment at the bottom of the chamber to achieve centrifugal separation due to the density difference. After that, increase the rotating speed to open valve2, so that the plasma can be delivered through an overflow channel to the collection chamber. The position of the overflow channel is pre-calculated. In general, the blood cells make up approximately 45% of the human blood. The position of the overflow channel to transfer the plasma in Figure 4.3 (d) is pre-defined for the collection chamber. A recession structure in the side channel is to capture the possible blood cells.
Figure 4.2 (a) Fluidic design of the plasma extraction system (b) Side view of the arrangement of the microfluidic disc and the actuation disc

4.3 Experimental result and discussion

Figure 4.4 illustrates the schematic diagram showing the operational sequence of the extraction process and the corresponding images of the prototype plasma extraction system in operation. In brief, the human blood was first loaded. At a spinning speed of 800rpm, the capillary valve opened and the blood was transferred to the metering chamber to define a volume of 10μL blood to be analysed. At a spinning speed of 1800rpm, valve 1 was opened and the blood was introduced to the sedimentation chamber while valve 2 remaining closed. Next, the disc was spun at 2500rpm for 10min to trigger the sedimentation of blood cells due to the density difference. After that, the rotating speed was increased to 3000rpm to open valve 2, the plasma was then delivered through the side channel into the collecting chamber. In Figure 4.4 (d), the actuation disc was removed for observation purpose. Figure 4.5 shows the curve for the spinning speed at different stages. The image of the disc after the extraction process was finished is shown in Figure 4.6. It is clear that
the plasma was successfully transferred to the collecting chamber. Figure 4.7 illustrates the microscope image of the blood cell sediment in the sedimentation chamber and the extracted plasma. It can be seen that most of the blood cells were removed.

Figure 4.3 working principle of the plasma extraction system. (a) load the blood, (b) meter the blood, (c) blood cell sedimentation, (d) collect the plasma.

Next, a series of experiments were conducted to investigate the influence of the sedimentation time on the purity of the plasma. Here we use the percentage of the removed blood cell to indicate the purity of the blood. The spinning speed of the sedimentation was around 3000rpm. It is apparent that the purity increases with the length of the sedimentation process. In the hospital, the process may take 10~15min under 6000rpm. With our proposed extraction system, the purity of the plasma can reach 99.5% in 6min. The main reason is that we only need to handle much less blood sample. In addition, due to the limit of our current configuration, the burst frequency of valve 2 can only reach 3000rpm which means the sedimentation spinning speed we
used is only about half of the one used in the clinic. As a result, the time for extraction can be further reduced by optimizing this system.

4.4 Conclusion

In this chapter, a plasma extraction system is successfully developed by implementing the sequential valves in chapter 3. This system is able to meter the blood sample and collect the extracted plasma. The inherent centrifugal force is employed for the blood cell sedimentation. The result shows that the purity of the plasma can reach 99.5% in 6 min. During the operation, the actuators of the valving system are isolated from the fluid system to avoid sample cross-contaminations. This prototype of the sequential valving system showed excellent ability to maintain durability at high speeds. It has very simple structure and the burst frequencies can be readily manipulated by simply adjust the preload of the supporting springs and the gap between the actuation disc and the microfluidic disc. The simple design and stable performance make it very easy to be integrated with other microfluidic functioning units for a wide variety and more complicated of lab-on-CD applications.
Figure 4.4 Schematic operation diagrams and the corresponding images of the plasma extraction system in the experiment.
Figure 4.5 Curve for the spinning speed at different stage

Figure 4.6 Image of the disc after the extraction process. The extracted plasma is transferred to the collecting chamber.
Figure 4.7 The microscope of (a) the blood cell in the sedimentation chamber and (b) the extracted plasma in the collection chamber.

Figure 4.8 The blood purity under different sedimentation time.
CHAPTER 5. MEMBRANE-BASED VALVES AND INWARD PUMPS FOR CENTRIFUGAL MICROFLUIDIC PLATFORMS

5.1 Introduction

Valving and pumping are two fundamental technologies for any fluidic system to perform accurate flow control. Prior researchers have developed various valves for centrifugal microfluidic systems. The most commonly applied valves are passive valves such as hydrophobic valve[3, 27], capillary valve[9, 29] and siphon valve[10, 15, 30-32]. They usually take advantage of the capillary effect and the property of the disc material to withhold the liquid. The drawback is that they require deliberate geometry design or surface modification. Besides, they are not suitable for the situation where sealing of reagents is necessary. In chapters 3, we have demonstrated the pinch type valve based on the spring plungers. Although it demonstrates good performance, the valve has its own drawback. The major problem is that the channel with a rectangular cross-section cannot be ideally closed. Besides, the alignment of the ball spacer increases the complexity of the fabrication process. As a result, these factors can bring uncertainty to the operation of the valving system and causes the inconsistent performance of the valves. Our experimental results show that the burst frequencies for one batch of valves can vary a lot. A new strategy is needed to activate the valves.

On the other hand, membrane-type valves with pneumatic control are very popular for the conventional microfluidic system. It usually is consist of a multi-layer structure. Typically a control layer made of elastic material such as PDMS is created adjacent to the fluidic channel. A pressure controller is connected to the control layer. When the external gas is pumped into the control layer the deflected membrane can close the channel. Hence, the “on/off” state of the valve can be controlled by the pneumatic pressure. This working principle can be implanted into the centrifugal microfluidic system with our novel flyball governor. The pneumatic pressure can be
replaced by the spring force supplied by the spring plungers embedded into the auction disc as described in chapter 4.

On the other hand, it is notable that intrinsic centrifugal force provides a universal fluid propulsion mechanism. However, the unidirectional flow from the center to the rim of the disc can also be regarded a limitation, since the radial path is limited by the radius of the centrifugal discs. Therefore, the inward pumping becomes necessary to propel the liquid from the rim of the disc back to the center. This can be used to provide the freedom to fluidic design and reagent storage for complex biological assays. Most of the prior researcher focus on the pneumatic expansion [33-35]. The drawback lies in the inconvenient actuation method and relative low pumping efficiency. Recently some new pumping methods have been proposed. Salin[36] utilized the compressed gas to pump the liquid backwards. But the external gas may cause contamination to the sample on the disc. Aeinehvand[37] integrated a small latex made balloon onto the disc. The balloon expanded and stored the elastic energy at high spinning speed and released the energy to pump the liquid to the center of the disc at low spinning speed. However, the complex structure adds complexity to the fabrication of the disc which consists of seven different layers. A simple, robust and non-contact pumping approach is still in need.

5.2 Design and concept

5.2.1 Working principle of flyball governor

The basis of our newly introduced valving and pumping mode is a specially designed flyball governor system. The actuation is performed by a specially designed flyball governor system described in the previous chapters. Figure 5.1 depicts the actuation of the proposed flyball governor. The microfluidic disc is installed on the top. The underlying flyball governor consists of actuation disc with spring plungers, linkage, flyball and a supporting spring. At the initial state,
the spring plunger is pressed against the microfluidic disc (Figure 5.1a). As the system starts to spin, the centrifugal force is exerted on the flyballs and in turn pulls the actuator disc downwards along the shaft through the linkages. The system can arrive at a new equilibrium when the pulling force balanced by the spring force produced by the supporting spring (Figure 5.1b). The displacement of the actuator disc is manipulated by the rotating speed. Figure 5.1c depicts the cross-section view of the spring plunger. The depth of the nose allows for easy movement with an external load.

![Figure 5.1 Schematic of the actuation of the flyball governor system. (a) At the initial position, the spring plungers are compressed against the microfluidic disc. (b) When the flyball governor is activated at high rotating speed, the actuator disc is driven downwards to pull the spring plungers away. (c) The cross-section view of the spring plunger.](image)

### 5.2.2 Concept of the membrane valving system

The operation of the membrane valve is based on the interaction between the spring plunger and the flexible covering membrane. The membrane valving system utilizing the three-layer design is shown in Figure 5.2 (a). Three identical valving units were integrated into one disc. The microfluidic disc consists of two PDMS layer and one PMMA disc. Figure 5.2 (b) gives the geometry of a single valve design. The top PDMS layer contains the loading chamber and
pneumatic chamber, while the bottom PDMS layer holds the valving chamber and collecting chamber. The pneumatic chamber and the valve chamber is connected by a 1.5mm diameter connection hole drilled in the PMMA disc. The valve structure is comprised of the pneumatic chamber, connection hole and the valve chamber. Figure 5.3 illustrates the working principle of the proposed valve. The spring plungers are embedded in the actuator disc. Their flat tip are used to apply pre-set forces to press the membrane of the valve chamber and seal off the connection hole and block the flow. As a result, these valves are initially at the “normally closed” states (Figure 5.3a). As the platform spins, the flyball governor is activated. The actuator disc with the spring plungers is pull down. The nose of the spring plunger is 1/4inch long. At low spinning speed, the spring plunger can still provide a strong compression force to seal the connection hole and block the flow (Figure 5.3 (b)). As the spinning speed increase to a critical value (burst frequency), the membrane can be recovered and the liquid can flow through the connection hole and reach the chambers in the bottom PDMS layer (Figure 5.3(c)). As a result, the on/off state of the valve can be purely controlled by the rotating speed.

Figure 5.2 Schematic of the microfluidic disc design for the valving system. (a) Exploded view showing the assembly and positions of three layers. (b) Schematic of a single valve on the disc. Both the loading and collecting chambers are 6mm in diameter and 500 μm in depth and the cross-section of the channel is 300×500 μm.
Figure 5.3 Cross-section view of the membrane valve with arrows to show its working principle. (a) The valve is initially closed. (b) The flow stops at the valve. (c) The valve is opened.

5.2.3 Concept of the inward pumping system

The function the inward pumping system is based on the valving system. The microfluidic disc still in the three-layer form and the fluidic design is shown in Figure 5.4. Figure 5.5 indicates its operational principle. When the valve is open at high rotating speed, all the sample can be delivered to the outward chamber C. Next the rotating speed is gradually reduced, so the actuator disc moves up and press the bottom PDMS layer. A compression chamber B is added next to the valve. A cylinder pin (6mm diameter, 1mm smaller than the compression chamber) is fabricated on actuator disc right beneath chamber B. More importantly, the tip of the spring plunger and the pin are set at different levels (d=4mm). Accordingly, the valve chamber will be compressed first to secure an airtight seal over the connection hole. When the compression chamber is squeezed subsequently, the resulting pneumatic pressure will push the sample from chamber C inward to chamber D and eventually reach chamber E as the spin continues.

5.3 Material and methods

5.3.1 Fabrication of the microfluidic disc

In brief, the microfluidic disc was fabricated in four steps as shown in Figure 5.6. The first step is to fabricate the SU-8 master mold with soft lithography method. A 500 μm thick layer of SU-8 100 photoresist (MicroChem, USA) was first spin-coated on a 4-inch silicon wafer.
Figure 5.4 Schematic of the microfluidic disc design for the inward pumping system. (a) Exploded view showing the assembly and positions of three discs. (b) Schematic of a single pump on the disc. All the chambers are 6mm in diameter except chamber B is 7mm in diameter. The radial distance between chamber C and D is 21mm. The cross-section of the channel is 300×500 μm.

Figure 5.5 Schematic of the inward pump with arrows to show its working principle.

Next fluidic patterns on a mask were transferred to the photoresist by UV exposure followed by the soft bake of photoresist. After the post bake and development, the mold was obtained for the PDMS casting. 8g PDMS (Sylgard184, Dow Corning, USA) was mixed in a 10:1 (m/m) of base / curing agent ratio and casted on the master mold. After being cured at 85°C in oven for 1hr, the PDMS layer was subsequently peeled off the mold and the holes were punched using sharpened blunt needle. The top and bottom PDMS layers can be obtained in the same way. In this study, we chose the 4-inch PMMA disc as the substrate. The center hole and connection
holes were drilled by CNC machining. After the PDMS layers were ready, the three parts were aligned and directly bonded together. The air bubbles were removed by the gentle touch. The results showed that the direct bonding between the PDMS and PMMA was strong enough to fulfil the requirements of our experiment and no leakage was observed during the operation.

![Diagram of fabrication process](image)

Figure 5.6 Schematic of the fabrication process for the microfluidic disc

5.3.2 Experimental setup

Figure 5.7 (a) shows the photo images of the assembled valving system. The system is equipped with a brushless motor with controller (BLDC-38S, Zhengke Motor Co, China) to spin the discs through the coupling. A LED tachometer monitors the rotating speed. The speed of the motor is controlled by pulse width modulation. The flyball governor comes with three identical flyballs, whose weight can be adjusted conveniently. A metal tube was placed in the center of the actuator disc so that the flyball governor can slide up and down freely along the shaft. The spring plungers with body diameter 1/4 inch and nose diameter 1/8 inch (part no.6423a12, Mcmaster, USA) were inserted into the actuator disc. They can provide a maximum nose force as 0.9lbs when compressed. Figure 5.7 also displays the layout of the valving and inward pumping system at rest. We used red food dye as the fluidic sample. The actuation of the system is demonstrated in Figure
5.7 (d). It is clear that the actuator disc is drawn away during the rotation from the microfluidic disc with the spring plungers and the supporting spring is significantly compressed.

![Image](image-url)

Figure 5.7 Photo images of the proposed microfluidic system. (a) Side view of the assembly of the system. (b) Close-up view of the normally closed valve. (c) Close-up view of the inward pumping system. (d) The actuation of the flyball system at 1000rpm. The actuator disc is moving down and compressing the supporting spring.

5.4 Results and discussion

5.4.1 Characterization of the membrane valve

Figure 5.8 presents the operation of a proposed valve. The valve was closed initially by the spring plunger. First 10μL red food dye was pipetted into the loading chamber. As the disc was spun at low speed, part of the sample liquid was pushed into the pneumatic chamber due to the interaction between the pressure of the trapped air and the induced centrifugal force exerted on the liquid. The valve stayed closed as the connection hole was still sealed. Next when the spinning speed exceeded the burst frequency, the compression of the spring plunger was removed and the liquid can pass the connection hole and reached the collecting chamber. The experimental results showed that 3%~9% of the sample was detained in the pneumatic chamber, which can be considered as the dead volume of this valve. The loss can be further minimized by optimize the geometry of the pneumatic chamber and its relative location to the connection hole.
Figure 5.8 Illustration of one cycle of the valving process. From left to right are the side view, top view and video snapshot of the valving system in action.

Figure 5.9 Measured burst frequency of the valve varies with (a) mass of the flyball, (b) preload of the supporting spring
Next a series of experiments were carried out to study the burst frequency of this valve. The burst frequency of the membrane valve subjects to many factors. Here we only investigated the most influential from the external mechanical system: the mass of the flyballs and the preload of the supporting spring (the vertical position of the microfluidic disc). The results are shown in Figure 5.9. It shows that lighter flyball and greater preload of the supporting spring can lead to higher burst frequency. The burst frequency can range from ~950 RPM to ~1600 RPM which offers flexibility in the design of the centrifugal microfluidic devices. The response time of the valve is around 1~3s depending on the acceleration of the motor. Additionally, this valve can be properly arranged to realize sequential or individual control as the spring plungers can be set at different levels on the actuator disc. The pre-load for each spring plungers can be defined individually.

5.4.2 Characterization of the inward pump

The operation of the proposed inward pumping was detailed in Figure 5.10. As outlined earlier, the function of the inward pump is based on the membrane valve. The burst frequency of the embedded valve was ~1000 rpm. Figure 5.11 describes a complete rotating speed cycle programmed for the inward pumping. First 10μL red food dye was injected. The valve was closed and the compression chamber was completely compressed. Next during the acceleration of the motor to 1600 rpm in 30s, the valve was slowly opened and the suction effect caused by the expansion of the compressed chamber helped to transfer the sample to the outward chamber C. Then the speed was maintained at 1600 rpm for 10s to empty the loading chamber. Next the rotating speed was slowly reduced to 0 in 30s. The valve was closed first and then the pin starts to trigger the pneumatic pressure. The transient pressure in the outward chamber was almost doubled. The experimental result showed that the liquid in the outward chamber C was delivered to the
receiving chamber in 2s. The flow rate for the current design was determined as 4.2 μL/s. The image shows that almost all the liquid in the outward chamber was successfully propelled 21mm backward to the inward chamber in one cycle. The pump efficiency for one cycle is close to 100% if we neglect the loss in the valve. In general, this membrane valve aided pump demonstrates outstanding capability and offers many advantages. It can be simply fabricated and operated without the external energy source. Moreover, we also replaced the membrane type valve with a pinch type valve with the pinch type valve demonstrated in Chapter 3. Their burst frequencies are comparable. The snapshots are shown in Figure 5.12. The fluidic design and the actuation system is the same as the previous inward pump. It turns out that the pinch type valve based inward pumping system required two cycles to complete the same task. The main reason is because of the leakage caused by the pinch type valve when the compression chamber is pressed and result in a compromised pneumatic pressure. The comparison further proves that the membrane type valve is capable of better sealing.

5.5 Conclusion

In conclusion, a new valving technology combining the conventional pin valve and flyball governor is successfully designed, constructed, and tested for application in centrifugal microfluidic platforms. In addition to the intrinsic advantages of the pin-type valves, its burst frequency can be easily manipulated by adjusting the parameters of the mechanical system without changes in the fluidic pattern. This type of valves comes with many advantages such as simple structure, robust operation, low dead volume and leakage, and non-contact with the sample. In addition, this new valve is capable of multiple valving operations and bears the high potential for comprehensive assay integration, automation and parallelization on the disc. Sequential control of multiple valves can be easily realized. The successful application of the valves in plasma
extraction proved the potential usefulness of the valve for a wide variety of biological/medical/chemical applications.

Figure 5.10 Illustration of the inward pumping. From left to right are the side view, top view and video snapshot of the pumping system in action.
Figure 5.11 Demonstration of the speed of the motor for a complete inward pumping cycle.

Figure 5.12 The sequential snapshots of the inward pumping by using a pinch-type valve: (a) The sample is loaded in the loading chamber (b) The valve is open to pass the sample to the outward chamber. (c) The liquid is propelled to the inward chamber after one cycle. (d) The sample reaches the inward chamber after the second cycle. (e) All the sample is delivered to the collecting chamber.
CHAPTER 6. A RAPID MICROMIXER FOR CENTRIFUGAL MICROFLUIDIC PLATFORM

6.1 Introduction

Micromixing is a critical process in miniaturized analysis systems for microfluidic devices. However, the mixing of fluids at the microscale faces a big challenge because viscous effects dominate at small scales, where the flow is laminar, and the mixing between different streams in the flow mainly depends on the molecular diffusion. Unfortunately, the diffusive mixing is slow compared with the convective mixing; thus the mixing length for molecular diffusion is always prohibitively long which negates most of the benefits of miniaturization. In recent years, to reduce the mixing time, many efficient chaotic micromixer have been explored. The concept of the chaotic mixer is to generate chaotic advection via stretching and folding fluids. Generally, micromixers can be broadly classified as two types: passive micromixers and active micromixers. In passive micromixer, the flow field is perturbed by changing the geometry of channels or adding geometric obstacles such as the square-wave micromixer[38], the zigzag micromixer[39], and the staggered herringbone micromixer[40]. In active micromixer, fluids are always perturbed by using an external energy source such as mechanical pulsation[41], acoustic vibration[42], magnetic force[43], electrohydrodynamic force[44] and electroosmotic force[45]. These reported strategies can improve mixing performance for conventional microfluidic system to some extent.

In the past several decades, centrifugal microfluidic has emerged as an important branch of the microfluidic system. The liquid mixing also become problematic for this micro system. However, the formentioned approaches cannot be easily implemented for centrifugal microfluidic platforms. First, the passive mixer usually requires high pressure due to the resistance in the fluidic channel. In the centrifugal microfluidics, the pressure exerted on the liquid can only be generated by the centrifugal force as the disc is rotating. It is not practical to reach the similar pressure in
most cases. Besides, for the active mixer, the enteral energy sources are difficult to be integrated with centrifugal microfluidic platforms. Considerable effort therefore has been dedicated to the development of the micromixing for centrifugal microfluidic systems. The most common method is known as “shake-mode mixing” which is to continuously alter the rotating direction of the disc[14]. The action of the liquid due to the frequent reversal of rotational direction can improve the mixing effect. However, a consequence of shake-mode mixing is that the disk is momentarily stationary when the rotational direction is altered which can possibly affect the operation of other unit on the disc such as the priming process for the siphon valves. Some other methods have also been reported. Noroozi et al[46] generated a reciprocating flow between two chambers for a PDMS chip. But the mixing time is not satisfying. Kong et al[47] blew compressed gas into the mixing chamber and agitated chaotic mixing. The limitation of this method is that the external gas may contaminate the sample liquid.

In order to create a more efficient pumping method, we describe a noncontact, easily implemented technique which allows for pumping the liquid from the rim of the disc to the center position. This technique provides a way to pump the fluids while spinning without complex disc design or fabrication process. It enables many sequences of operations. This unit operation has been demonstrated in a fluidic test module that has been fully characterized.

In this chapter, we developed a simple micromixer on the centrifugal microfluidic platform. This approach relies on the flyball governor system described in the previous chapters. As the disc rotate, a pin attached to the flyball governor system enables to periodically pressurize the membrane of the mixing chamber to introduce the chaotic flow to enhance the mixing performance.
6.2 Material and method

The operation of this technique is also based on the fly-ball governor system developed in previous chapter. Figure 6.1 shows the schematic diagram of an assembled platform. It is similar to the valving system introduced earlier. The difference is that the pin is printed with the actuation disc together. The spring plunger is no longer needed here. The pin is used to press the mixing chamber at low spinning speed as shown in Figure 6.1a.

![Schematic of the mixing system](image)

Figure 6.1(a) Schematic of the mixing system. It consists of three parts from top to bottom: the microfluidic disc, actuation layer and the flyball governor system. The mixing chamber is compressed initially. (b) Demonstration of the mixing system at high spinning speed. (c) Schematic of the disc design. The PDMS layer consists of three identical patterns. The microchannel is 300 μm high and 300 μm width. The fabrication of microfluidic disc is completed by standard photolithography and PDMS casting.

Figure 6.2 gives the functional principle of this mixing system. It is generally accomplished in three phases.

1. **Load the sample:** At the initial state, the sample liquids are introduced to the loading chambers.

2. **Liquid pumping:** Increase the rotating speed to deliver the liquids to the chamber.

3. **Chaotic mixing:** After the mixing chamber is filled, periodically alter the rotational speed to drive the pin to move up and down, accordingly. The fluids in the mixing chamber then can be agitated to cause chaotic mixing due to the elasticity of the PDMS membrane.
6.3 Results and discussion

To verify the mixing efficiency of the mixing performance, operation of the mixing system is shown by the images in Figure 6.3. 50μL of red dye solution and 50μL of DI water were introduced into the loading chambers. The red dye is on the right and the DI water is on the right. A complete cycle is shown from Figure 6.3a to d. The mixing chamber was initially compressed. The disc was first spun at 800rpm to first remove the compression on the mixing chamber and propel the liquids into the mixing chamber as shown in Figure 6.3b. Next, the spinning speed decreased to 600rpm so that the pin pressed the chamber again to cause rapid mixing (Figure 6.3c). When the pin was driven away, it is clear that the liquids were better mixed in Figure 6.3d. Figure 6.4 shows the corresponding spinning speed during the operation. The cycle can then be repeated until the liquids are completely mixed.

Figure 6.5 gives the sequential images obtained by the wireless camera demonstrating the effectiveness of the mixing after four mixing cycles (t=15s). During each cycle, the pin was capable of squeezing and stretching the fluids. Thorough mixing appears to be obtained in the end.
Figure 6.3 Implementation of the mixing system on the centrifugal microfluidic system : (a) The samples were loaded. (b) The samples were delivered to the mixing chamber. (c) The mixing chamber was compressed by the pin connected to the flyball governor at low mixing frequency. (d) The pin was released at high spinning speed.

To quantify the mixing performance, we use the standard derivation of the pixel intensity to represent the state of the mixing. After each cycle, the image of the disc was captured and stored. For each image, the area containing the mixing chamber was cut out as the region of interest. Then these images were imported into Matlab. The gray intensities for all the pixel were obtained. Hence, the standard derivation for each images can be directly calculated. The smaller standard derivation stands for better mixing. Figure 6.6 shows the histograms and the standard derivations for each cycles. It can be seen that the grey-scale distribution of the images becomes more uniform during the mixing progress. It also need to mention that due to the limitation of the resolution of the camera and the irregular shape of the liquid, the results may be inaccurate to some extent. But the standard derivation still can be used as an indication of the mixing performance.

To evaluate the effectiveness of this mixing strategy, the same amounts of fluids were transferred into the mixing chamber and spun with the disc for 15s. The mixing is based on pure diffusion. Figure 6.7 shows the diffusive mixing result. Most of the liquids stayed unmixed. The
standard deviation is 0.25. It can be seen that the reciprocating compression mixing can greatly enhance the mixing performance compared with pure diffusive mixing.

![Diagram showing spinning speed and time phases](image)

Figure 6.4 Curve for the spinning speed during the mixing process

![Images of liquids](image)

Figure 6.5 Images of the liquids after (a) one mixing cycle (b) two mixing cycles (c) three mixing cycles (d) four mixing cycles

Further we investigated the effect of the operational parameters. First, the rotational speed of disc was adjusted. Our experimental results showed that the pin was just moved away from the microfluidic disc to perform the “release” operation at 800rpm. The higher rotating speed was not helpful to deliver better mixing performance due to the extra longer deceleration process needed for the “compress” operation. Therefore, the highest rotational speed of disc was set to 800rpm.

The previous experiment was conducted in a “compress and release” fashion as shown in Figure 6.4, where the pin repeatedly compressed the mixing chamber. Next the rotation pattern
was changed by adding an extra “holding phase” which means the compression process lasted for a while and then was released. As seen in Figure 6.8, the extra holding phase did not improve the effectiveness of this mixing performance. In the holding phase, the mixing only relies on diffusion. The results indicated that the improvement of the mixing effect was mainly caused by the chaotic mixing rather than the diffusive mixing.

Figure 6.6 Evaluation of the mixing progress for four sequential mixing cycles. As mixing continues, the distribution of pixel intensities shifts towards unity in the histograms.

Figure 6.7 Gray-scale image of the mixing chamber after the disc was spun after 15s. The standard derivation is 0.25.
Then the effect of the pin size was studied. An 8mm pin was used to replace the 4.2mm pin. The comparison is demonstrated in Figure 6.9. It can be observed that the mixing performance was improved. The main reason is that the larger pin can produce a stronger compression effect to boost the chaotic mixing. Additionally, the thinner streams can increase the contact area between the two fluids and thus increase the diffusion. But the size of the pin is limited to the size of the chamber and the volume of the sample. As a result, to change the pin size is an effective way to improve the mixing performance for this mixing system and an optimum pin size can be found for the mixer.

This mixing technique works on the chaotic mixing caused by the external mechanical system and the primary advantage of this mixing method is that it does not requires extra entities integrated into the disc such as beads or magnets. Thus this method can effectively avoid any possible contact contamination. Besides, this mixing also relies on the function of the flyball governor. So the valving or inward pumping units developed in the previous chapters can be integrated with this mixing system to achieve complex fluid handling on centrifugal microfluidic platform. One possible drawback of the mixing method is that it is not compatible with cells or beads because they may be damaged during the compression process. Nevertheless, this mixing method can provides an efficient, non-contact way of mixing liquids on centrifugal microfluidic platforms, adding another possible way for the existing mixing approaches.

6.4 Conclusion

In this chapter, we explored the application of the flyball governor in the centrifugal mixing. When the flyball governor moves up and down, the mixing chamber is periodically pressed to achieve effective chaotic mixing. Compared to diffusive mixing, this mixing method enable uniform mixing of liquids in a short time. It can be used alone and integrated with other flyball
governor based actuated device such as valves and inward pumps developed earlier for multi-
operation sequences. Therefore, it bears great potential for more complicated of lab-on-CD
applications.

Figure 6.8 Comparison of the standard deviation of the mixing performance with and without the
holding phase.

Figure 6.9 The standard deviation of the mixing performance with different pin size
CHAPTER 7. SUMMARY AND FUTURE WORK

7.1 Summary

In this dissertation, a series of fluidic functions on centrifugal microfluidic platforms were designed, fabricated and tested. They all worked on an integrated flyball governor for the rotatory platform. This research work produced fruitful results. It proposed three kinds of valves, two types of inward pumps, a functional plasma extraction system and a rapid mixer. The first one is based on the magnetic force and the second one is on the basis of spring force and the last one is a membrane valve. All valving system demonstrate good control of the fluid movement. The latter two valves are capable of sequential control. It proves that the flyball governor system is very compatible with centrifugal fluidic technologies. The major advantage of this new actuation technology is that its burst frequency can be conveniently manipulated by adjusting the parameters of the mechanical system without changes in the fluidic pattern. As a proof of concept, a sequential valving system capable of metering and centrifugal sediment was developed for plasma extraction from whole blood. The resulting residual cell concentration was less than 0.5%. Next, two types of inward pumping systems were designed and tested. The result shows that both the inward pumps were capable of the pumping over a radial distance of 21mm in a short time. It thus improves the usage of space on the disc and paves the way to interconnect several functional units. In the end, a micromixer was developed based on the similar principle. The results show that the flyball governor system can effectively agitate the chaotic mixing of the sample liquids by periodically deflecting the PDMS membrane of the mixing chamber. The mixing effect can thus be enhanced.

In the proposed Lab-on-CD instrument, all the components on the microfluidic chip of the Lab-on-CD system are passive without power supplies, and therefore can be inexpensively fabricated using micro-replication technology. The flyball governor and servo-motor are the permanent components of the instrument, while microfluidic disc that contacts test samples can be
disposable. This design helps to eliminate the contaminations and cleaning requirements for the instrument. The simple design and stable performance make it very easy to be integrated with other microfluidic functioning units for a wide variety and more complicated of lab-on-CD applications.

7.2 Future work

Although the research demonstrated in this dissertation has made contribution to this dynamically evolving field, more efforts are still in need to improve the fabrication technique and device development.

1) In this research work, all the microfluidic disc were fabricated using soft lithography method. This fabrication method is currently only suitable under laboratory conditions. For the purpose of industrial-scale process, the microfluidic disc can be fabricated using other methods. For example, the disc for the membrane based valve can be fabricated in a multi-layer structure. The channels and chambers can be engraved on plastic disc by laser or CNC method. An elastic layer such as tape or rubber paper can serve as the membrane for the actuation. Then all the layers can be bonded together to form a compact disc.

2) For the proposed plasma extraction system, the spinning speed for the blood sedimentation is relatively low compared with the centrifuge used in the hospital. The burst frequency for the valves can be further increased to ~6000rpm or higher by adjust the mechanic parameters of the flyball governor. In this way, the extracted plasma can be further purified in a shorter time. Besides, here we only demonstrate the plasma extraction which is only the first step for medical diagnose. The sequential detection units can be added into the system to enable quick identification of a disease and lower cost of diagnostic and testing. We expect that this centrifugal device can be part of the point-of-care system.
3) In this research work, the flyball governor was employed to accomplish most of the functions. Although it is reliable and useful, it can only move in the vertical direction and thus provide limited actuation approaches. The electromechanical components can also be integrated into the spinning platform through the slip ring and provide more freedom to the fluidic control. For example, the solenoid can be integrated into the actuation disc to close the channel or stir the sample liquids for better mixing.
REFERENCES


VITA

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