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Integration of Stable Droplet Formation on a CD Microfluidic Device for Extreme Point of Care Applications

THESIS

submitted in partial satisfaction of the requirements
for the degree of

MASTER OF SCIENCE

in Engineering

by

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Thesis Committee:
Professor Marc Madou, Chair
Professor William Tang
Professor Zhongping Chen

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DEDICATION

This work is first dedicated to my sister Archana Ashok for being my true mentor through my graduate program. Her honest criticism and encouragement allowed me to give my best.
My parents for teaching me to "Scale the pinnacle of my dreams one base-camp at a time".
My grandfather(R.I.P Mr. Sivasubramanian) for his immense faith in me which motivated me to always follow my passion.
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ABSTRACT OF THE THESIS

Integration of Stable Droplet Formation on a CD Microfluidic Device
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By

Shruthi Vatsyayani Ganesh

Master of Science in Engineering

University of California, Irvine, 2017

Professor Marc Madou, Chair

With the advent of microfluidic technologies for molecular diagnostics, a lot of emphasis has been placed on developing diagnostic tools for resource poor regions in the form of Extreme Point of Care devices. To ensure commercial viability of such a device there is a need to develop an accurate sample to answer system, which is robust, portable, isolated yet highly sensitive and cost effective. This need has been a driving force for research involving integration of different microsystems like droplet microfluidics, Compact-disc (CD)microfluidics along with sample preparation and detection modules on a single platform. This work attempts to develop a proof of concept prototype of one such device using existing CD microfluidics tools to generate stable droplets used in point of care diagnostics (POC diagnostics). Apart from using a fairly newer technique for droplet generation and stabilization, the work aims to develop this method focused towards diagnostics for rural healthcare. The motivation for this work is first described with an emphasis on the current need for diagnostic testing in rural health-care and the general guidelines prescribed by WHO for such a sample to answer system. Furthermore, a background on CD and droplet microfluidics is presented to understand the merits and de-merits of each system and the need for integrating the two. This phase of the thesis also includes different methods employed/demonstrated to generate droplets on a spinning platform. An overview on the detection platforms is also presented to

understand the challenges involved in building an extreme point of care device. In the third phase of the thesis, general manufacturing techniques and materials used to accomplish this work is presented. Lastly, design trials for droplet generation is presented. The shortcomings of these trials are solved by investigating mechanisms pertaining to design modification and use of agarose based droplet generation to ensure a more robust sample processing method. This method is further characterized and compared with non-agarose based system and the results are analyzed. In conclusion, future prospects of this work are discussed in relation to extreme POC applications.

Chapter 1

Motivation

1.1 The Need for Extreme Point of Care Devices

The primary step in prevention and cure of diseases involves the diagnosis of the disease itself. In the current setting, disease diagnosis is achieved in a proper laboratory setting involving the patient visiting the doctors office. The patient then undergoes various tests and the samples are processed and analyzed in off-site laboratories. The turnover time for these tests is at least 1-2 hours and even more when sent off-site. With the advent of point of care technologies prototypes have been made to replace conventional bench-top techniques using microfluidics. Now, in resource poor-settings, point-of-care technologies(POCT) used in developed countries do not work as the implementation challenges are accentuated by the lack of basic amenities like water, very intermittent power supply and low affordability. Thus sample and reagent storage is difficult and the sample processing environment harsh. In developing countries there is an uneven distribution of available healthcare. Trained personnel and laboratory facilities are concentrated in urban regions. Clinics in resource poor settings lack facilities to conduct diagnostic tests and the skilled manpower to interpret

Parameter	Problem	Effect on healthcare
Power	Fluctuating Voltage, Frequent Power Cuts	Most benchtop equipments cannot function
Environmental Conditions	High Dust, Humidity, Temperature	Reagent Storage is difficult.
Supplies	Lack of Diagnostic tests like urine dipstick, printers etc.	Servicing of equipment would require long travels and cost more.
Human Resource	Lack of literacy and health education, unskilled manpower	Lack of Healthcare professionals in rural regions who can conduct diagnostic tests and treatments

Table 1.1: Challenges in EPOC

Cost Type	Cost Incurred in Indian Rupees
Consultation Cost per visit	$Rs.200$
Pathological Test	$Rs.800$
Transportation Fee per visit	$Rs.30$
Number of visits	4
Total Cost	$Rs.1720$

Table 1.2: Cost Incurred for Disease Detection-Survey Conducted in December 2016

these tests. This leads to transport of samples to laboratory centers making it unaffordable, time consuming and furthermore compromising sample quality. Figure 1 represents the three-tier healthcare set-up in India[39]. Sub-centers form the primary point of contact for the rural population and are the only form of clinics easily accessible. These health centers are equipped with dealing pre-natal and post-natal care and the ability to check the patient's vitals alone. Therefore, microfluidic technology geared towards providing diagnostic facilities in these centers is the need of the hour. This forms a premise for the motivation to develop tailored POC tests for resource poor settings. A brief overview is presented on the problems of implementing rapid and accurate diagnostics in rural health kiosks in the table 1.1

A brief survey was conducted for this work to analyze cost incurred for treatment of infectious diseases like Tuberculosis and Malaria. An example from the same is of Person A with a monthly income of $Rs.8500$ or 128 USD approximately, supporting a family of 5. His expenditure for Malaria diagnosis test is listed in the table 1.2 From the survey results in

Table 1.2, the person A has spent more than his monthly income for diagnosing malaria. People in these resource poor regions most often do not get detected for the disease owing to the time, travel and cost involved.

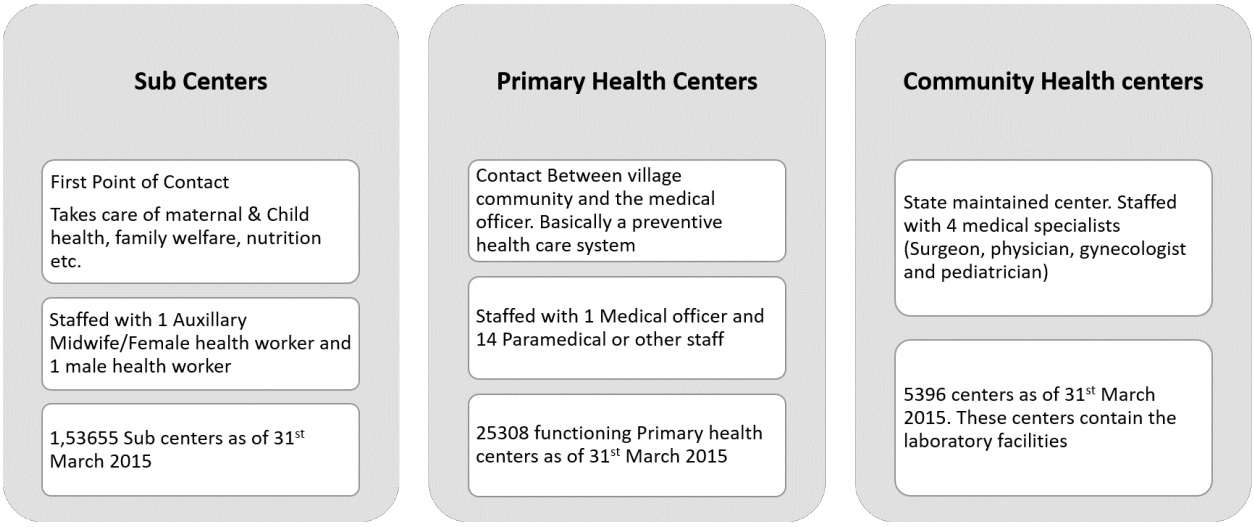


Figure 1.1: 3-Tier Healthcare System in India

1.2 The Ideal Extreme POC Device

Although a strict guideline doesn't exist in low earning economies pertaining to the manufacturing of these devices, a criterion has been set up by WHO to which a POC device should conform. For healthcare technologies geared towards developing nations, these prove to be the very challenges. ASSURED criteria stands for Affordable, Sensitive, Specific, User-friendly, Rapid and Robust, Equipment free and Deliverable to End-Users[27]. Comparing the potential of the existing technology and this criteria, a rough sketch of an ideal E-POC device can be derived. POC devices primarily employ microfluidics fundamentals. This method utilizes low sample and reagent volumes bringing down the prohibitive cost of reagents. The use of disposable cheap plastics like polycarbonate like in the CD technology further lower the cost and manage to maintain sample quality with a single run per disposable disc. The use of these transparent plastics and of elastomers like PDMS enables use of sensitive optical detection methods. Development in microanalytical techniques has further demonstrated high sensitivities using small volumes through now common techniques like capillary electrophoresis, high-pressure liquid chromatography[40]. Microfluidic systems especially droplet based have been used for high-throughput yet highly accurate assays. Microfluidic tests use small volumes increasing the heat and mass transfer rates yielding rapid turnover time for tests. Inferring from the available plethora of research work in microfluidics, an ideal E-POC technology would be a complete sample to answer system. It would have incorporated techniques to store reagents and prepare samples (like a world-to-chip interface). The microfluidic disposable would then contain all the sample processing steps typical of a molecular diagnostic device like lysis, mixing, DNA amplification, etc. The detection system employed should be less bulky and not require external equipment. This system should have high selectivity and sensitivity towards the target species. Furthermore, this device should also have a method to collect the test results and store them enabling integration into a centralized patient history database. All these phases must be part of a

single isolated system. This would be portable, rapid and robust. The integrated system should also be modular in nature to enable easy troubleshooting. The technologies integrated to build this single platform must be compatible for a desktop manufacturing setting. This enables the diagnostic tool to be more patient driven and eventually the operation of this device would not require the use of skilled manpower. CAD models of the microfluidic device could be easily sent across and manufactured at the source based on the demand. This platform should have the flexibility to provide on-disc heating and cooling as well, expand the range of assays that could be run on this single platform. In short, a single system equipped with temperature control, detection, sample and reagent storage, data storage and wifi compatibility, would be a permanent set-up in the rural clinic setting. The disposable disc for testing various infectious diseases or the sample to be tested would then be run on this permanent platform. Lastly, these platforms should be reliable enough to foster telemedicine such that even doctors in urban region could use the analysis from these tests to provide further treatment to the patient. Although this ideal device is not fully developed and commercially viable, research and funding efforts have been made to stem work in this field. This work is one such attempt focusing on the disposable microfluidic device tested on a proof-of concept prototype platform designed towards Extreme POC applications.

1.3 Generic Process Flow of a Diagnostic Device

A complete sample to answer system would involve a combination of sample preparation steps followed by the actual analysis. Sample preparation involves concentration and Lysis of the sample. The analysis involves the Polymerase Chain Reaction assay(PCR) followed by detection using optical or electrochemical methods. Figure 1.2. represents a flow chart of the various process steps in a typical molecular diagnostic tool.

These steps may vary with specifics of the assay being conducted on the disc. A brief understanding and examples of each of the process steps is provided as a tool to conceptualize a disposable microfluidic device.

Sample Preparation

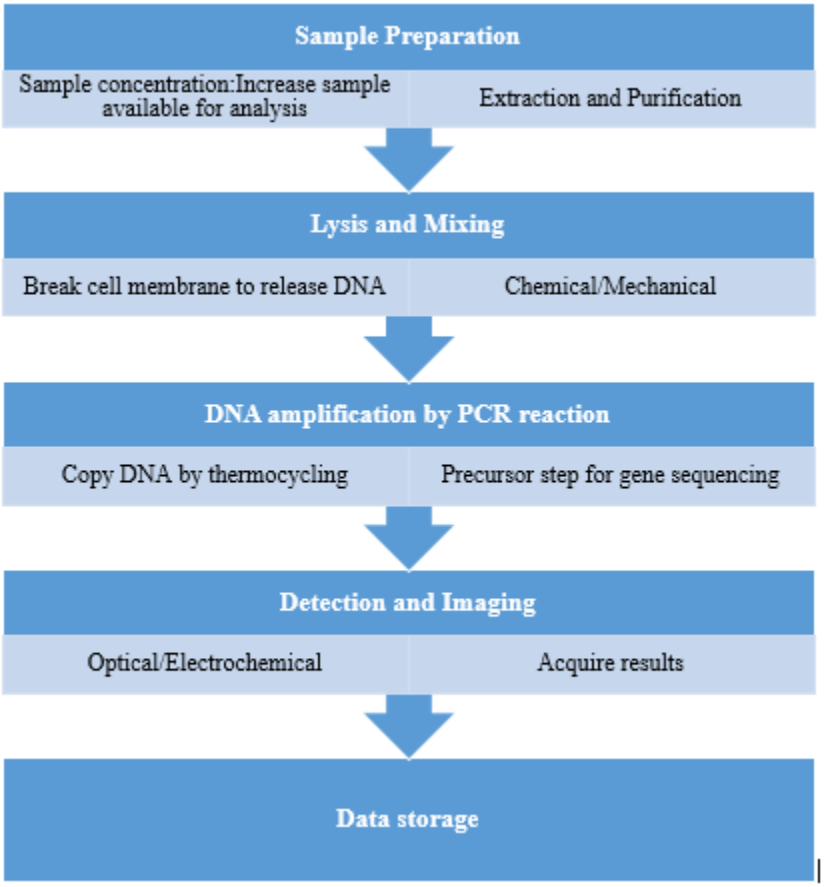


Figure 1.2: Process Flow of a Diagnostic Device

PCR is one of the most commonly used assays in molecular diagnostics. It is a simple yet an elegant way to pick a piece of DNA of interest and copy it. It is highly sensitive and only a low concentration of DNA is required to make multitude of copies. Sample preparation therefore becomes a vital step to maintain purity of the sample by removing inhibitors. Furthermore, samples obtained are by nature dilute. Therefore pre-concentration of this sample is key to make available enough DNA concentration for replication. Newer methods to do PCR are based on limiting dilution which involves sample preparation as a key step. Figure 1.3 below shows the amount pre-concentration of dilute samples to enable detection.[1]

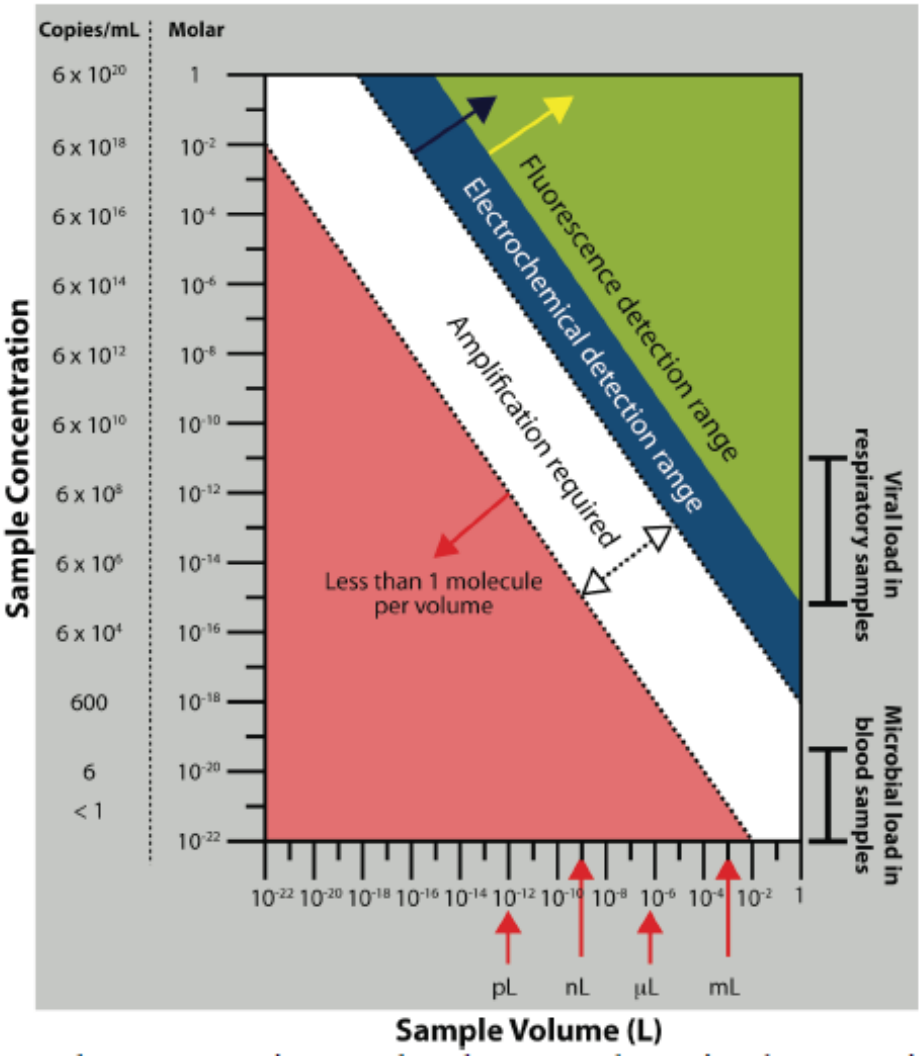


Figure 1.3: Reguired sample concentration for molecular diagnostics.

The effect of sample preparation on the quality of the reaction has been illustrated like detection of *T. gondii* [8],fungal cultures [25] and Hepatitis B virus[20] , to name a few.
Ethanol precipitation, phenol-chloroform extraction and silica mini column adsorption are some of the benchtop methods to extract and purify samples prior PCR.
Conventional sample preparation steps are complex and time consuming. It is challenging to scale them down to a microfluidic device albeit necessary to have an effective sample to answer system.In conventional systems, multiple centrifugation steps are employed to extract and purify the sample. In microfluidic testing, the sample first undergoes a separation step followed by cell lysis to discard impurities and then to make available the target DNA. [17]

Lysis

A subset of sample preparation steps, Lysis entails the disruption of the sample cell walls using chemical,thermal,electrical or mechanical methods to make available DNA for the diagnostic steps. Discussed briefly below is the extraction of DNA using mechanical,chemical and thermal methods which are usually easier to employ on an E-POC device.
Most common chemical lysis method uses buffers like proteinase K, sodium dodecyl sulphate, Triton X-100 [41]. Chemical lysis involves solubilizing the cell membrane using buffers. It is a clean method to incorporate, selective and does not damage protein. However, from the point of view of E-POC devices, reagent storage is a challenge to be overcome. Mechanical lysis uses shear or frictional forces or even compression to physically disrupt the cell wall. An example is the work by Di Carlo et.al. The work describes a process of passing the cells over "nano-scale barbs" fabricated using Bulk Etching and Wafer Bonding [6]. Another method is the usage of microfluidic channel geometry to deform the cells to break the cell membrane. Demonstrated on a PDMS based devices where one side of the narrow channel is curved while the other is flat. Cell solution is loaded into flow channel followed by loading of water in the control channel after the cells adhere to the walls.The pressure is regulated externally. The PDMS contracts due to pressure from the control channel. [41] Thermal lysis utilizes high temperatures to extract DNA material. An example of this is microcantilever heaters

which provide local resistive heating for lysis [33]. Integration of sample preparation on a CD Microfluidic Device is discussed in depth ahead.

Polymerase Chain Reaction(PCR)

The prepared sample is amplified for the detection steps. This is done either through PCR or reverse transcriptase PCR if the sample of interest is in the form of RNA(Ribonucleic acid). Here a general understanding of the process is presented through a brief background on PCR in order to identify engineering challenges involved in manufacturing the sample to answer platform. A general PCR reaction requires the target or sample DNA, primers(short strands of DNA) which are designed based on prior knowledge of the target DNA, ddNTPs(Di-deoxynucleotides) which act as chain terminators,DNA Polymerase, and nucleotides[31]. A typical PCR reaction requires 30-60 cycles wherein the temperatures are cycled between 95° to denature DNA, 60° for the primer to bond and 72° for annealing. Mullis' work has been pivotal in bringing rapid progress in molecular diagnostics. For a sample to answer system, the challenge lies in being able to control the temperatures precisely in the low power environment on a dispoable microfluidic device, maintaining the reaction in a pure and isolated environment and finally reducing the time of reaction and detection suitable for rapid testing. Further sections depict implementation of PCR on a CD. Alternate ways to isotheramlly replicate DNA has also been demonstarated in different works. Few examples include Strand Displacement Amplification, Helicase Dependent Amplification, Nucleic Acid Sequence Based Amplicfication amongst others. [1]

Electrochemical and Optical Detection

The detection method depends heavily on the analyte or rather to answer the question"what are you trying to detect?". For rapid testing there is need for the testing method to be sensitive, fast and to not require external equipment to make the device convenient and portable. Restricting this section to detection for rapid diagnostics, a lot of research has been done to incorporate optical or electrochemical detection on the microfluidic device. Based on

the outline of steps usually involved in rapid diagnostics, the most useful method would be to perform detection during the PCR reaction termed as "Real-time PCR" where fluorescent labels are bound to the double-stranded DNA for detection. An alternate method is to use DNA mircoarrays. DNA microarrays permit multiplexing as different fluorescent probes can be used to detect different analytes. An interesting example of electrochemical detection is the work by Edman et.al.[7]. The idea is to add an enzymatic label to a DNA probe and the products of the enzyme based reaction is detected electrochemically. Electrochemical detection utilizes cheaper hardware and can be bulk manufactured using fabrication methods employed in the semiconductor industry. However, the fabrication can be tricky and the detection system would be in direct contact with the sample unlike optical methods which capture fluorescence.

Arguably, electrochemical detection provides faster and sensitive detection(owing to miniaturization) and is independent of the material's optical properties making it a flexible system. Furthermore unlike optical detection, electrochemical detection does not maintain restrictions on sample volumes or sample properties like turbidity. Optical detection depends on sample volumes owing to the minimum required optical path length. [18]

Chapter 2

Theory and Background

2.1 CD Microfluidics

2.1.1 Physics of Operation

The three dominant forces on the CD due to spinning of the disc includes the Centrifugal force F_ω, Euler forceF_E and the Coriolis forceF_C. The centrifugal force plays a pivotal role in fluid flow manipulation by propelling the fluid forward radially. The Euler force becomes crucial when the angular velocity is changing with time. This force is perpendicular to the centrifugal force and opposite to the direction of angular acceleration. The Coriolis force is used extensively for particle separation and switching the direction of flow. It acts perpendicular to the velocity vector of the moving particle. The force balance equation including these pseudo forces is mathematically presented below:

$$\vec{F_{tot}} = \rho(\vec{\omega} \times \vec{r}) \times \vec{\omega} - 2\rho\dot{r} \times \vec{\omega} - \rho\dot{\omega} \times \vec{r} \tag{2.1}$$

where ρ is the liquid density, ω is the angular velocity in rad/s, $\dot{r}$ is the velocity vector of the particle moving on the disc, $\dot{\omega}$ is the angular acceleration, and r is the average distance of the liquid from the center of the disc. While all three forces are proportional to the mass of the particle, the Centrifugal force, represented by the first term in Eq. 2.1, is proportional to the square of the angular velocity, making it the strongest acting force in a rotating frame of reference.

2.1.2 CD Microfluidics as a Micro-Total Analysis System

CD Microfluidics has been extensively researched upon for the last decade as a candidate for point of care assays. Lateral flow Assays and Paper microfluidics dominate the cheap and rapid testing market but the Lab on Disc(LOD) system is efficient for molecular diagnostics requiring complex assay steps and sensitivity. The simplicity of manufacturing and using a LOD system alongside its portability and low cost make it an ideal candidate for Extreme Point of Care Applications. Furthermore, the CD system combats the most common problem in microfluidics-pumping and valving. The pseudo forces efficiently drive the fluid forward radially permitting various sample preparation and analysis steps to be performed efficiently overcoming air bubble formation. Most common steps of a diagnostic assay have been successfully implemented on the CD platform such as lysis, mixing and centrifuge and nucleic acid analysis. Background on how the generic processes required for molecular diagnostics are implemented on a CD is summarized as a table. Few example papers are also referred to for the reader's convenience. This table is not an exhaustive list of the capabilities of the CD but briefly refers to the physics of operation of the different functions and cites examples of implementation.

Function	Method of Implementation on a CD	Examples of implementation
Mixing and Separation	The coriolis force is used homogenize samples.Mixing maybe passive(geometry based) or active(external actuation).Valving is achieved using capillary force to balance the centrifugal force [12].Separation is done by exploiting the density differences in the sample and using the centrifugal force to sediment the denser substance	RBC separation from blood plasma [Kuo, JN. Li, BS] [21],Microballoon based mixing on a CD [28]
Mechanical Lysis	The lysis chamber contains ferromagnetic discs and ceramic or silica beads. The applied magnetic field moves the ferromagnetic discs. This in turn generates shear forces by moving the silica or ceramic beads	CD based system for cell-lysis [16]
PCR	Contact and Non-contact heating has been demonstrated on a CD. For contact heating, a disc shaped printed circuit board(PCB) is placed beneath the microfluidic disc. The disc is spun to reduce loss of reagent.Non-contact heating uses an IR lamp with a parabolic reflector to heat the sample chamber.	Contact heating using a thermoelectric element in a spinning PCB[15], Non-contact heating for PCR [30]
Optical Detection	A bead tag binds to a capture protein to which the sample protein binds. A laser diode or LED excitation source with a camera device could be used to measure intensity	Flurosocence detection implemented for digital PCR on a CD [37]
Electrochemical Detection	Microelectodes combined with a slip ring set-up provide electrical contact to the spinning disc[19]	Flow enhanced Amperometric sensing on the CD for the ELISA immunoassay [18]

Table 2.1: CD as a μ TAS

2.2 Droplet Microfluidics

Before diving into the genesis of droplet microfluidics, it is important to understand the need for such a system. Pertaining to the idea of building a cost effective, flexible diagnostic system, this section provides a brief understanding of the current PCR systems which are quantitative in nature. The idea of droplet digital PCR is presented followed by drawing conclusions on the need to integrate droplet microfluidics on the CD. Lastly a brief background on droplet generation methods focusing on the centrifugal microfluidics platforms is presented.

2.2.1 PCR Systems: Droplet Digital PCR

There are various types of Polymerase chain reactions like quantitative PCR, nested PCR(double amplification step for higher sensitivity), Real-time PCR(simultaneous detection and amplification), digital PCR(direct quantification), hot start PCR(reduced non-specific amplification, multiplex PCR(multiple targets detected simultaneously using multiple primers) etc. [29].[32] Broadly classifying, the first generation of PCR involved using gel electrophoresis. The results of the reaction were measured upon completion of the electrophoresis. This has a long turnaround time and is limited by the diffusion of DNA through the gel matrix. The second generation of PCR is the real-time PCR. The progress of the reaction is measured at the end of each cycle. A point on the fluoroscence curve depicts the increase of signal intensity over the background and this is marked as the measurement of the progress of reaction. A combination of limited dilution, poisson statistics and end point PCR lead to the inception of digital PCR [10]. This idea has been in progress for years and is based on having an end point which is binary, i.e. positive or negative on a sample which has one or more target molecules and yields a positive end point. Limiting dilution is applied by doing multiple replications at varying dilutions of the sample to be amplified. The end

result is computed using poisson statistics which determines the starting concentration.[38] To achieve this, the sample to be amplified is partitioned into replicate reactions at limiting dilution which yields an end point of 1 or 0 molecules in most reactions, i.e. amplified target or no amplified target. Microfluidics has been vital in implementation of digital PCR for the ease of enabling sample partitioning. Multiple channels split the sample into nanoliter volumes. Another novel method to achieve this is through forming emulsions of the sample leading to droplet based PCR.[11] Real-time PCR relies on the fluoroscence analog curve which is compared to a calibration curve. It is indirect and is difficult to reproduce. On the other hand droplet-digital PCR(ddPCR) provides absolute quantification but has a lower dynamic range. This can be improved by generating highly uniform emulsions [11]

2.2.2 Need to integrate Droplet and CD Microfluidics

Droplet Microfluidics enables development of high throughput biological assays by employing massive sample partitioning. Every single droplet is capable of acting like a micro-reactor in itself. This enables cost reduction in terms of reagent use, possibility of multiplexing and improved accuracy. Furthermore, encapsulation of the sample or reagent in a droplet ensures no contact between the sample and the microfabricated surface. This ensures high purity of the reaction.[24] The reduction in sample size also enables faster heat, mass transfer and shorter diffusion lengths which speeds up reactions and reduces turn-around time. Many chemical and biological assays also require particle encapsulation.Droplet microfluidics provides this function on a CD platform. CD microfluidics on the other hand can act like a completely isolated system without requiring bulky add-hoc devices. The physics of operation helps overcome the requirement of external syringe pumps and aides manufacturing a low cost isolated, miniature, robust bio-medical laboratory Integrating droplet microfluidics with a CD Microfluidic device would enable development of a versatile Extreme Point of care device capable of not only of a sample to answer system but also of genome based studies[5].

However integration of the two technologies does have challenges. The physics of droplet formation is not completely understood and is heavily dependent on the flow rates of both dispersed and continuous phases. The compounded complexity of the effect of centrifugal force on these flow rates adds to non-uniformity of the droplet reactors [2]

2.2.3 Methods to Generate Droplets on a CD

The method to generate droplets on a CD differs from lab on chip devices owing to the physics of operation of CD based devices. The centrifugal force acts on a unit volume of fluid whereas flow driven by external pressure(due to syringe pumps)are transmitted by the surface. Furthermore, on the CD the artificial gravitational force generated increases radially yielding higher flow rates [9]. Haeberle et.al. also demonstrated the fluidic conditions that would be required to generate droplets on the CD using dimensionless numbers. Majority of the droplet generation applications employ one of the three methods: T-junction, Flow focusing and Co-flow. With respect to the thesis, these methods are explained for water droplets in oil system. The oil would act as the continuous phase and water as the dispersed phase.[34]

T-junction

The oil and water phases meet in a T-shaped junction. The water phase upon reaching the continuous phase pinches off in the droplet form and gets carried in the oil stream. This is a fairly straightforward method and different flow regimes are attained based on the capillary number.

Co-Flow

Here the oil and water phases meet in parallel as shown in the schematic.The channel carrying the water phase is completely engulfed by the oil stream. This method is again dominated by the capillary number or rather the flow rates of the two streams yielding different flow

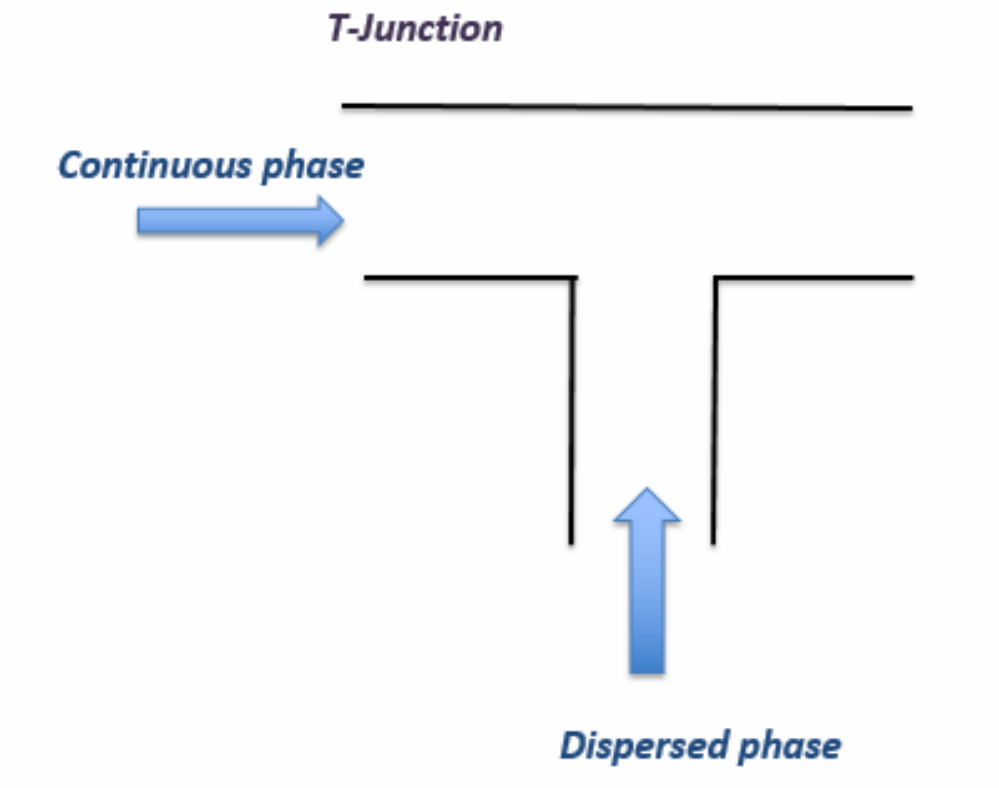


Figure 2.1: Schematic of T-junction geometry for Droplet Generation

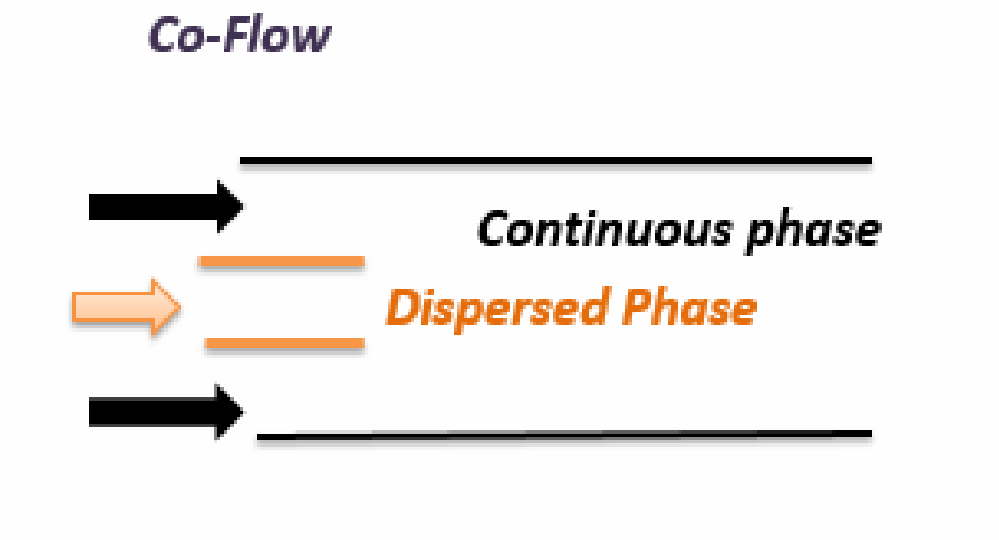


Figure 2.2: Schematic of Co-flow geometry for Droplet Generation

regimes.

Flow Focusing

This system is a modified version of the co-flow system and produces more uniform droplets than the T-junction and the co-flow systems.

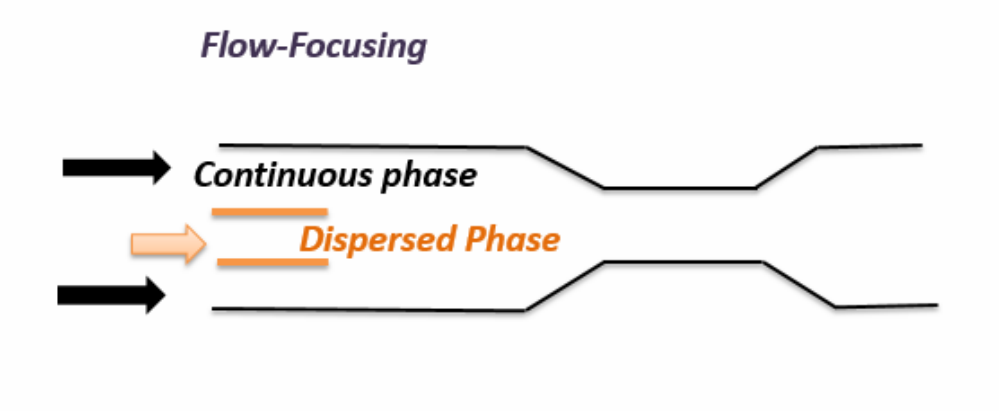


Figure 2.3: Schematic of Flow-Focusing method of Droplet Generation

The presence of the orifice structure in the parallel flow system creates different flow regimes based on the flow rates of the two phases. A tabular format of the different droplet generation methods is presented below.

Property	T-Junction	Co-Flow	Flow-Focusing	Step-Emulsification
Flow Regimes	Dripping, Squeezing, Parallel Flow	Dripping, Jetting, Parallel Flow	Squeezing, Dripping, Jetting, Parallel Flow	None
Flow rate of dispersed and continuous phase	Droplet size	Droplet Size	Droplet Size	Independent
Droplet Size range	Varied with flow Rates	Varied with flow Rates	Varied with flow rates	Fixed for given Geometry
Monodispersity	Difficult to attain	Difficult to attain	Easier to attain	Easier to attain
Implementation on CD	Difficult	Difficult	Difficult	Yes
Multiplexing	Difficult	Difficult	Difficult	Yes

Figure 2.4: Comparison of droplet Generation methods

Step-Emulsification

This method is slightly different from the other conventional methods in that it involves

only one flowing phase. The oil acts as a passive phase whereas the water as the moving phase. There are modifications to this design with multiple droplet forming units(edge emulsification). Schuler[34] in his thesis has described in detail the working of this method. The important components involves the droplet microchannel, the terrace and the chamber containing the passive phase(here, the oil). The entire system is first wetted with the oil phase. The water phase then flows into the chamber containing oil through the droplet microchannel. This microchannel defines the droplet size. The channel widens into a terrace region. This terrace region has a constant depth as that of the channel but is wider. The water phase at the end of the terrace meets a sudden backward facing step into the oil chamber. The spinning disc enables the oil to pinch the droplet that forms here. The terrace along with the step aides the droplet formation. This can be visualized using the Young Laplace equation:

$$\delta P = P_i - P_o = \gamma(1/r_1 - 1/r_2) \tag{2.2}$$

where δP refers to the pressure difference inside and outside of the droplet i.e. dispersed and continuous phase pressure difference. The radii r_1 and r_2 depicts the change in radius as the droplet grows in size when passing through the terrace and into the step region. r_1 grows until it reaches a value equal to half the channel depth. This leads to r_2 forming a necking region in order to satisfy the equation. Based on the figure 2.4, centrifugal step emulsification is an easier and more effective method to implement on the spinning platform since it is independent of flow rates and therefore can be multiplexed more effectively. With the conventional methods, it would be difficult to maintain constant flow rate in multiple droplet forming units.

2.3 Centrifugal Microfluidics Platform-CD Spinstand

The development of the CD microfluidic platform started with a clinical chemistry analyzer from the Oak Ridge National Labs by N Anderson. The next generation involved the Picollo rotor system wth integrated sample processing and reagent storage by Abaxis Inc. [1]. The first sample to answer system was outlined in the paper by Steigert et.al.[36]. The Gamera team along with Madou and Kellogg contributed heavily in developing the first centrifugal sample to answer system in 1998 [1]. The three generations of spinstand can now be classified into spinstands equipped with - slip ring to provide electrical contact, on disc wireless power transfer and lastly the spinstand capable of energy harvesting developed by Joseph et.al. [13]. The comparison of these three technologies have been discussed in the review paper by Smith.et.al [35]. In summary, the entire set-up represents a centrifuge wherein the brushless DC motor aims to propel the fluid. With advances in wireless power transmission, it is possible to incorporate active components on the set-up without using bulky ad-hoc equipment required for sensing and data storage. The concept of CD microfluidics for E-POC applications is attractive also owing to its compatibility to CD-ROM readers. It is therefore possible to convert a Discman or CD-ROM into a simple biomedical laboratory using the inherent laser for readout from the microfluidic disc.[35]

Chapter 3

Generating Stable Droplets on a CD Microfluidic Device

Key output success criteria for Droplet formation

- Uniform Droplet Size
- Droplet should be stable between $95°C$ and $25°C$ temperature range
- Droplet lifetime of 24 hours

Framework for Development

- Fabrication of the Microfluidic CD
- Selection of Emulsion recipe for droplet formation
- Trial Run of the recipe on the fabricated CD
- Review results- possible design modifications to troubleshoot issues

- Evaluate Droplet Stability under Applied Temperature
- Re fabricate the CD with necessary design modifications
- Re-run experiments to gather data

3.1 Materials and Fabrication:CD Microfluidic Device

3.1.1 Design Components for Droplet Formation

Droplet formation would occur when a dispersed phase would flow into the passive/continuous phase(depending on droplet formation method) at certain spin speed of the CD. The droplet quality,hence, would depend on optimization of the process parameters. To conceptualize a disc design to form droplets, a rough CAD sketch was generated comprising of the main components to enable droplet generation. 3.1 shows a drawing of the different components. The design tries to utilize the centrifugal force to the maximum and hence the chamber is drawn to lie radially from the disc center. The reaction chamber contains the passive phase. The dispersed phase flows through the droplet channel into the reaction chamber. The channel geometry would impact the droplet quality. As described in the background section, the chosen droplet generation method also requires a terrace region. This controls the droplet diameter as the dispersed phase flows into the chamber. Lastly, the venting system contains any back pressure and dead volume.

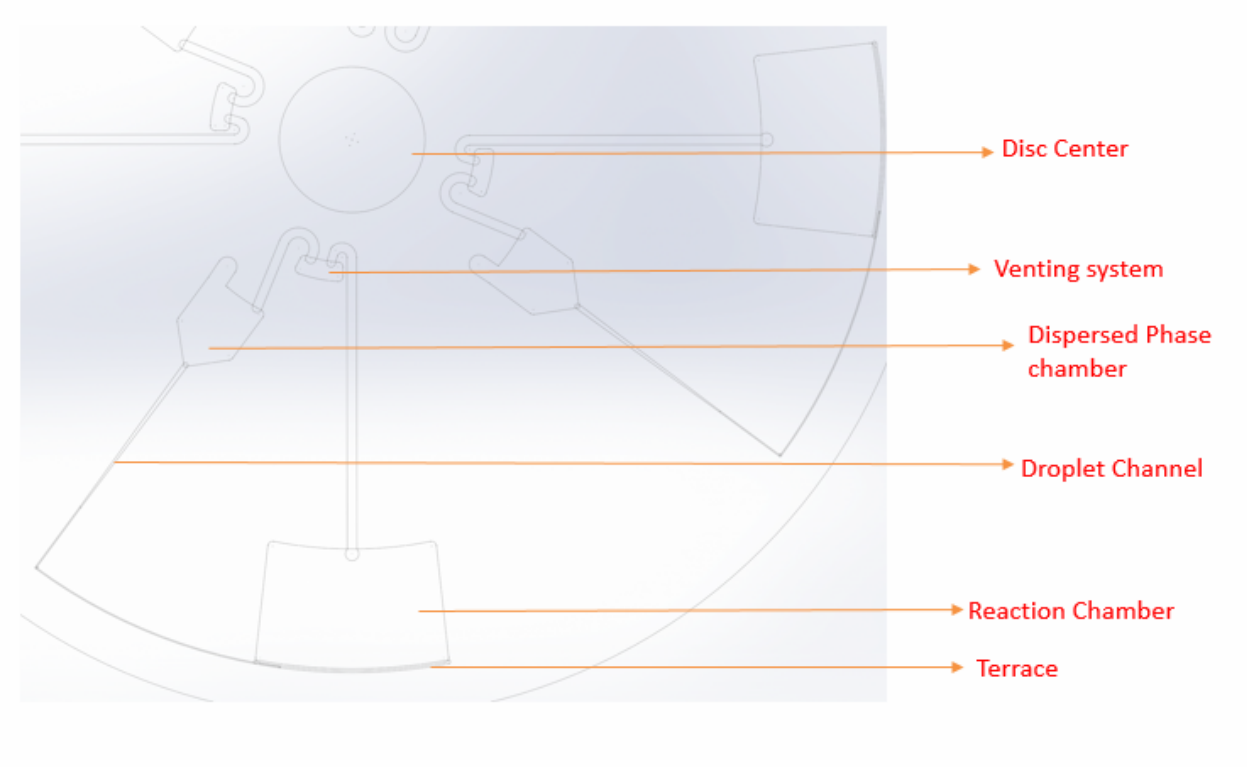


Figure 3.1: Drawing of components for Droplet formation

3.1.2 Fabrication and Prototyping

Available Prototyping Methods: Reasoning for chosen method

Choice of fabrication method depends heavily on the choice of materials and the commercial viability with respect to the target market. Becker et.al. have described chronologically the shift towards polymer based fabrication techniques in their comprehensive review. [4] Fabrication of microfluidic devices has borrowed heavily from the microelectronics industry-photolithography and etching (especially Deep Reactive Ion Etch)of silicon and glass. Silicon is fairly expensive and is opaque and thus eliminates the use of optical detection in most systems. Furthermore, the fabrication of these substrates demands the use of clean room environment, high voltage and temperatures. Lastly, the chosen microfluidic device should be disposable. This requires cheaper and easily available materials. The advancement in MEMS led to exploration of microfluidic devices using photolithography of thermoset resins

often used as photoresists. This yielded structures with relatively smaller z-height. The use of LIGA enabled manufacturing high aspect ratio devices. The development of SU-8 as a photoresist to simplify the LIGA process influenced the use of SU-8 as a primary material for microfluidics and BioMEMS applications. The resist is first spin coated on to the wafer. UV exposure is applied using a photomask to create channels and other features. A second layer of resist can also be added as a sacrificial layer. Post exposure baking yields strong cross-linked SU-8 structures. This process is slow and cumbersome. Similar to using silicon fabrication, this method also requires clean room equipment. The use of polymers like PDMS(Poly dimethyl siloxane), plexiglass, acrylic, polycarbonate, etc. for microfluidics is preferred owing to their mechanical flexibility, cost, ease of processing and a less expensive scale-up method. [14] PDMS is an elastomer and undergoes volume changes under application of mechanical or thermal stresses. PDMS is used extensively to manufacture microfluidic devices using soft lithography. The basic idea is to use PDMS stamps to make microfluidic structures. These molds can be manufactured using traditional lithography techniques. Microfluidic channels can be printed using PDMS stamps by micro transfer molding, micro contact molding among other techniques[14]. In case of Biological applications like E-POC, sample volumes of around 20 μL is required. Hence, the traditional microfabrication techniques may not work. A combination of macro and microfabrication techniques would hence be required to create these molecular diagnostic devices. Furthermore, the cost constraint in lithography methods is further accentuated by the "terrace" based design used for droplet generation. This design would mandate a two step-lithography processes which can be avoided using mechanical or thermal based subtractive techniques. [26] For this purpose to build the intended E-POC device, two prototyping methods can be explored : CNC Milling and Laser etching. The biggest constraint in both methods includes the material of choice. For example, PMMA(Polymethylmethacrylate) can be easily prototyped by laser cutting but being a brittle material, it is not very suitable for milling. Polycarbonate on the other hand can be easily milled and is robust and scratch resistant. However, it releases

toxic by-products on being laser cut. If the material were compatible with both methods, laser cutting would be the obvious choice in terms of turn around time. However, the major drawback with laser cutting includes heat affected zones(HAZ) wherein the removed material may resolidify altering the material properties. The HAZ maybe reduced by altering the laser parameters like pulsating the laser source, reducing the laser wavelength and so on. [26] Furthermore, a lower wavelength improves the ablation process.

$$DOF = \frac{(1.27\lambda)}{NA^2} \tag{3.1}$$

where, NA is the numerical aperture of the lens system and λ is the wavelength. The results of laser beam and that of a light source is compared in the book by Dr. Madou [26] and a more detailed explanation regarding heat affected zones is also provided. Laser beams, in conclusion have a better DOF than resolution. CNC milling provides a rough finish and a poor resolution. However, this method posses no material restrictions. The smallest feature size would depend on the drill bit size and strength. Both Hot Embossing and Injection moulding can be used as replication methods. The key is to not have to resort to lithography or milling to make parts and use these time consuming methods only for making the master part.[22] Poly-carbonate is chosen owing to its robustness, scratch resistance, flexibility of using optical detection on the platform and the cost.

Prototyping Process flow

The lab-scale prototyping process is a 3-step one and is an adapted version of Laminate Object Manufacturing. The CAD file is used to mill the disc. For this work, the CNC used is Roland Modela MDX-40. The milled polycarbonate disc is cleaned and de-bured(polycarbonate generates powdered residue making the cut rough) using a exacto knife.The sample loading holes are then punched(using Drummel Mill). A heat-resistant single sided adhesive(Flexcon)

is used to cover the chambers. This is crucial since the disc undergoes thermal cycling to conduct PCR. A second, double-sided, pressure sensitive adhesive is used to bind this disc with a bottom plastic disc. An exploded view of this assembly is presented below along with the images of the prototyping machine used for the process. After the assembly, the disc is passed between pressure rollers to ensure proper sealing.

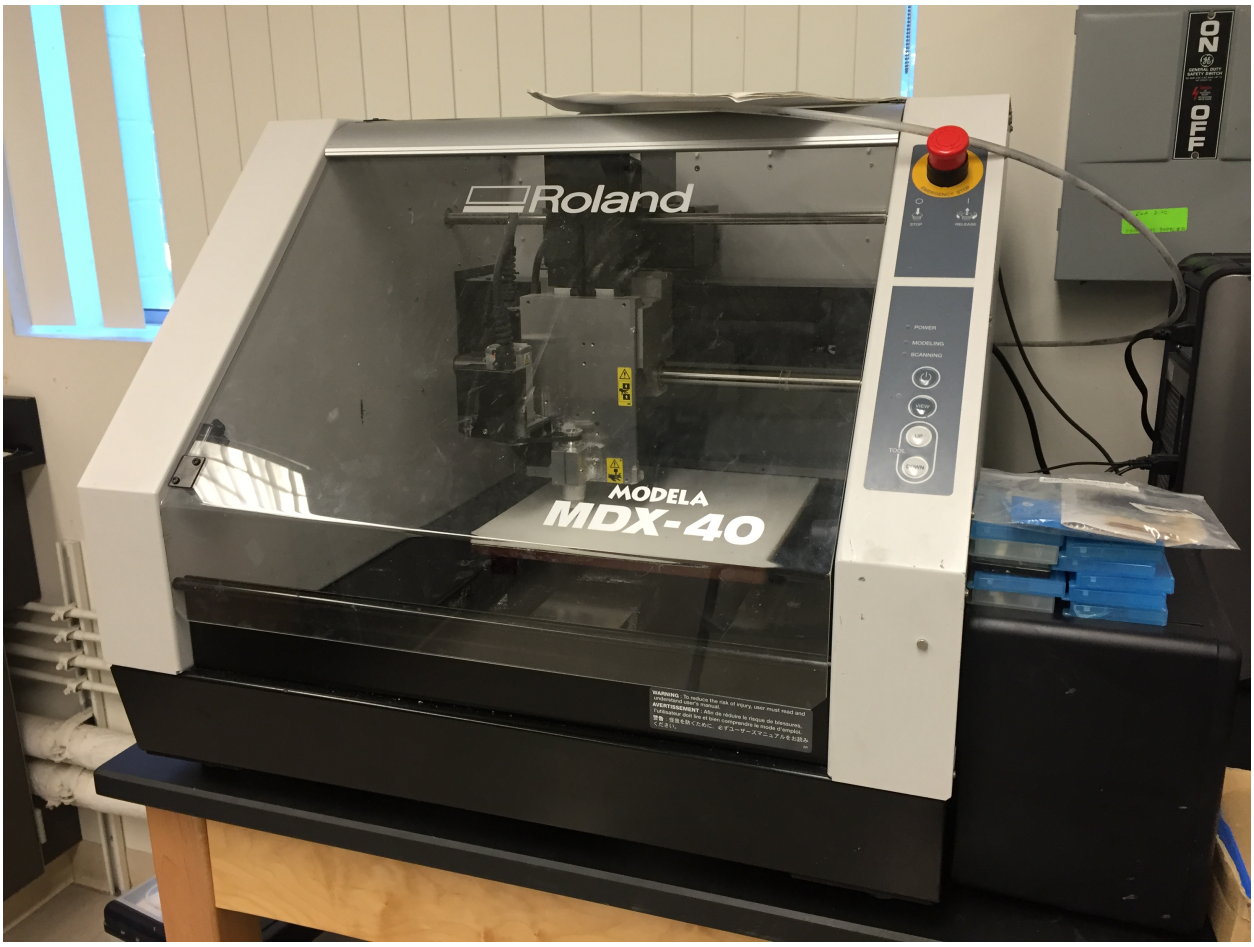


Figure 3.2: CNC mill

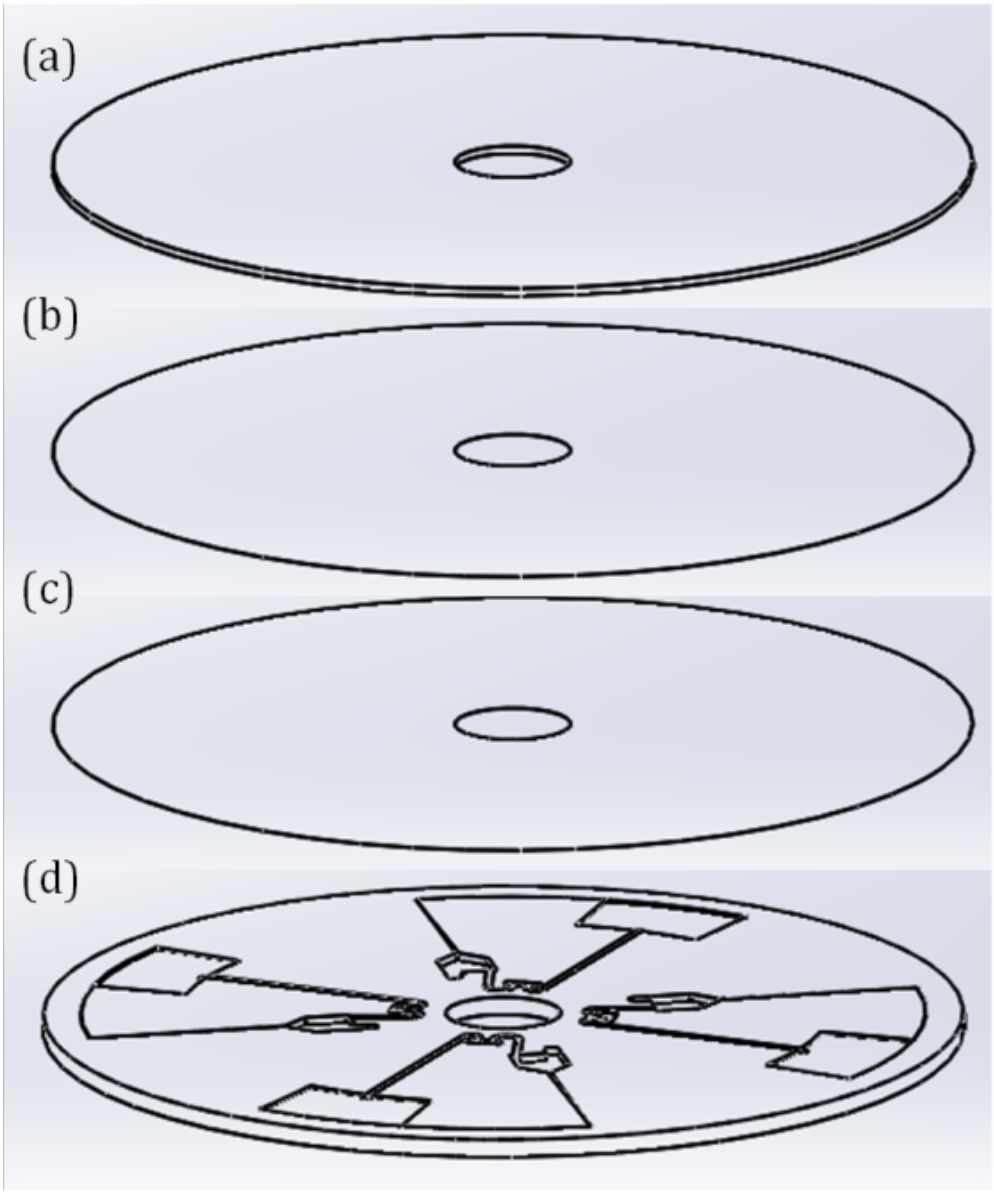


Figure 3.3: Exploded view of the assembled polycarbonate disc

3.2 Experimental Set-up

The set-up contains three main components:The microfluidic disc itself forms the disposable component, the spinstand(operated using a simple brushless DC motor) is compatible to run different disposable components; in this case one disc at a time and lastly the IR system which is further divided into the IR lamp to heat the disc; an IR sensor to provide feedback on the temperature and a PID controller to monitor the temperature and to enable thermal cycling.

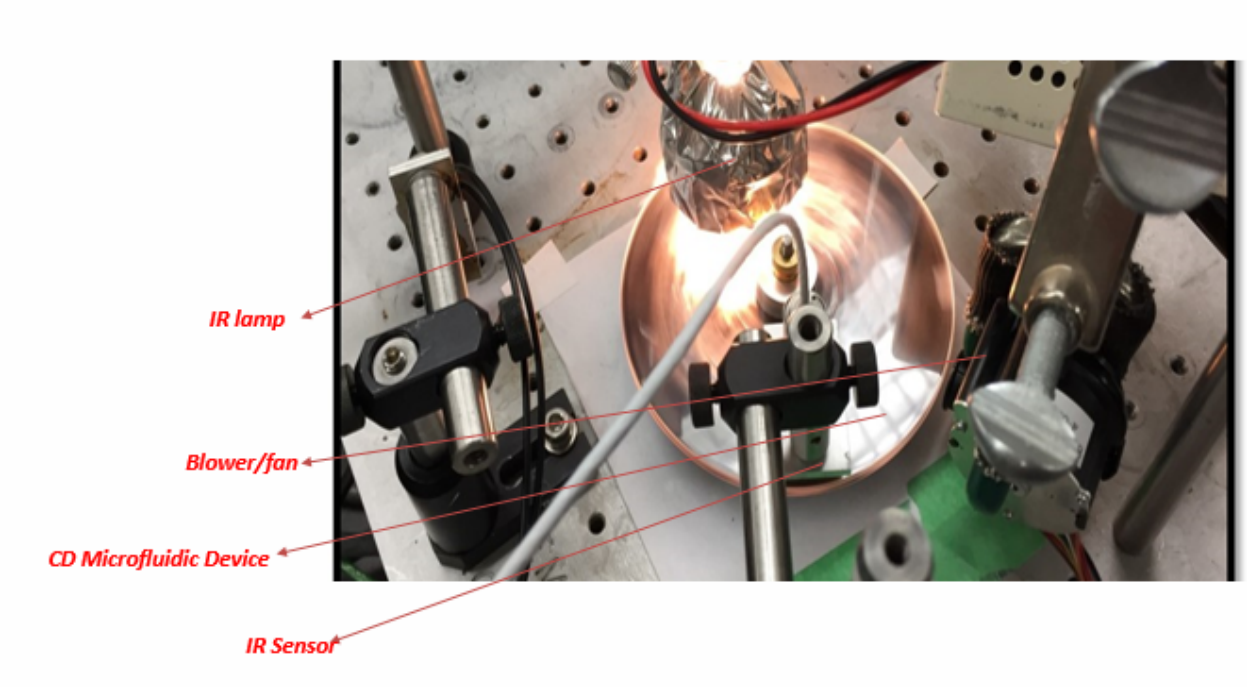


Figure 3.4: Experimental set-up

3.2.1 Steps for Generating Droplets on the Disc

The Disc is loaded onto the spinstand and the spinstand is powered up. The oil is first pipetted through the sample holes. The disc is spun for $60s$. This enables wetting of the channel walls with the oil. This makes the channel walls even more hydrophobic and thus

the dispersed phase would not adhere to the walls. Meanwhile, the mastermix/dispersed phase is prepared and is pippetted through the sample holes and the disc spun for $120s$. This enables droplet formation. The droplets float on the passive phase or the oil phase. The success of the process is determined by gauging the diameter uniformity of the droplets and their stability under applied temperature. Once the droplets are formed, PCR is carried out. For this, the IR lamp is switched on along with the IR sensor. The IR lamp is placed to focus the light onto the chamber containing droplets. The sensor is positioned to read temperature at the same point.The distance of the IR sensor from the chamber is a third of the distance between the lamp and the disc surface. This is precisely maintained to avoid damage to the polycarbonate surface due to incorrect temperature readings. Based on the assay requirements(which depends heavily on the design of the test in terms of how long the DNA would take to denature and the reagents added for PCR reaction) the temperatures are set. For this particular test, the droplets are first heated to 95° for $10min$ followed by alternating the temperature between 95° , 60° and 72°. The droplets are held at 95° for $30s$ followed by 60° for one minute. This is carried on for 40 cycles following which the droplets are cooled to below room temperature. General PCR reactions may vary from this procedure in terms alternating the three temperatures such that the droplets are first held at 95° followed by 60° and finally 72°. To enable detection, the droplets may contain a fluorescent dye. For testing purposes, the experiments here included only droplets and colored dye and the stability of droplets under temperature was tested by making note of change in average droplet size and number of droplets before and after PCR. To ascertain the functioning of the entire set-up, one batch of ready made Bio-Rad droplets were made to undergo PCR on this set-up and the fluorescent images were gathered to verify the success of the reaction.

The figure above (3.5) shows how the droplets form when the disc is spun. The details regarding the emulsion recipe is discussed in the further sections.

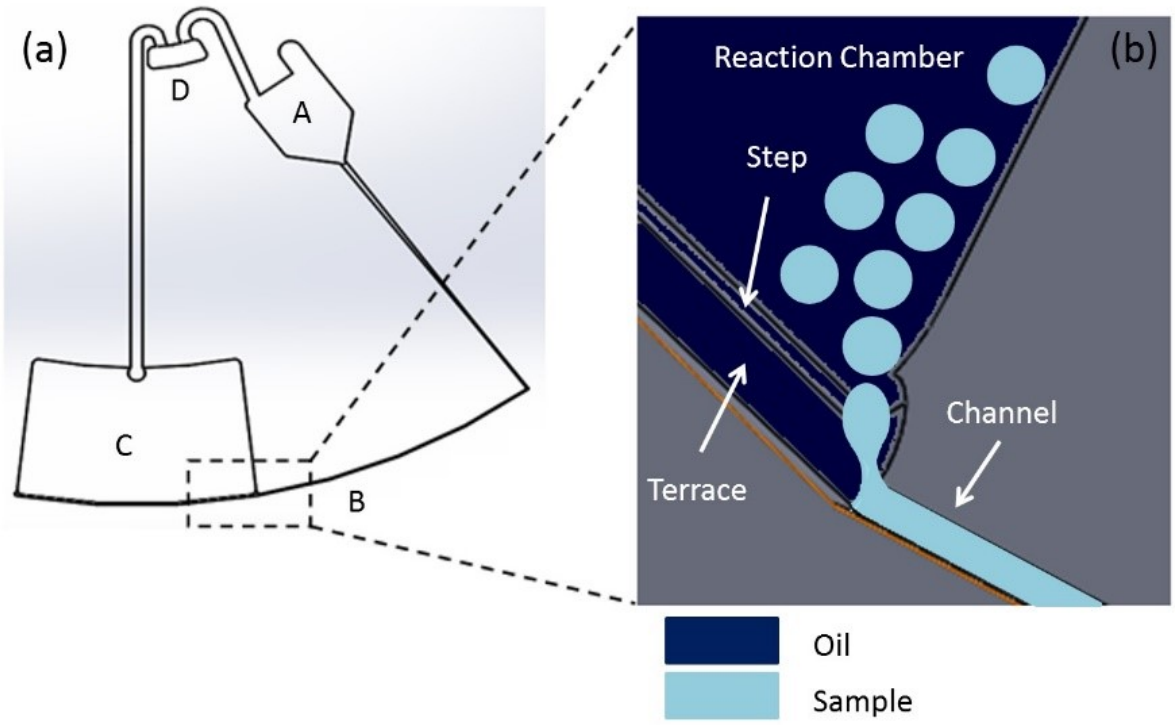


Figure 3.5: Enhanced schematic of droplet formation

3.3 Materials:Droplet Formation

As discussed in the previous chapter, the sample forms the dispersed phase. For the first part of this work, an off-the shelf PCR mastermix diluted with water was used. The mastermix was purchased from Bio-Rad. It has a density of 1.267g/mL. A colored dye is added to enable visualization of the droplets. A number of common oils were tried to analyse the best oil-mastermix combination. Furthermore, based on the initial rough CAD sketch, four preliminary designs were tired to test the droplet quality. These screening experiments were carried out in parallel to find the best design-emulsion combination.

3.3.1 Oil-Design Combination

4 discs were milled with different geometries to define the following features

- Master mix flow from top or bottom, a function of emulsion recipe
- The droplet channel entering into the chamber either from the side or center

Therefore the design trials can be categorized into the following:

- Trial 1 : Master mix flows from the top center

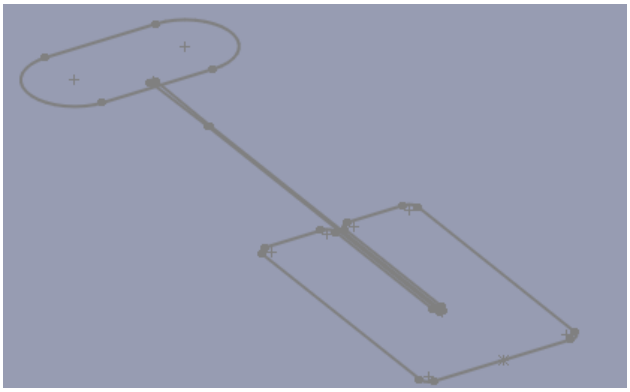


Figure 3.6: Trial1

- Trial 2: Master mix flows from the bottom center
- Trial 3: Droplet formation channel on the top from the side
- Trial 4: Droplet formation channel on the bottom from the side

Oil having different densities were tried to optimize the droplet formation. These are represented as below(3.1)

Oil	**Density**	**Observation**	**Design Trial Implemented**
Silicone oil	$0.96 g/mL$	Almost negligible droplet formation	Trials $1, 2, 3, 4$
Fluorinert	$1.85 g/mL$	Worked	Trials $1, 2, 3, 4$
Bio-Rad	$1.58 g/mL$	Worked Best	Trials $2, 4$

Table 3.1: Oil-Design Combinations tested

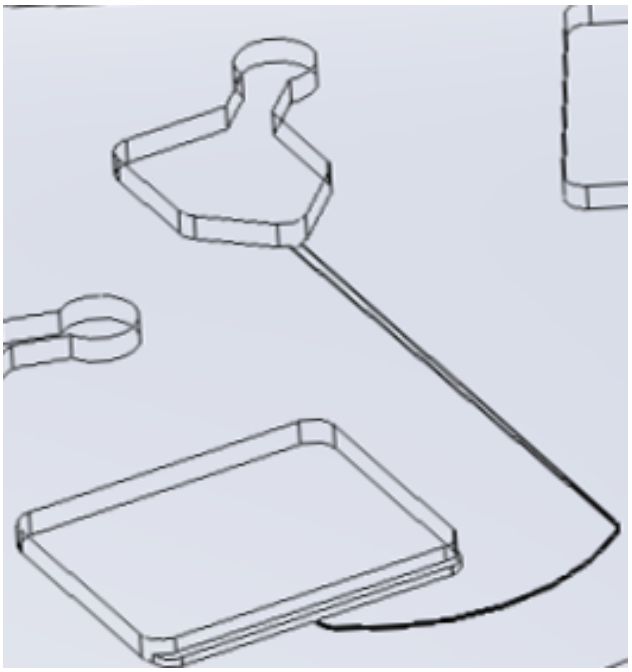


Figure 3.7: Trial2

Figure 3.8: Trial3

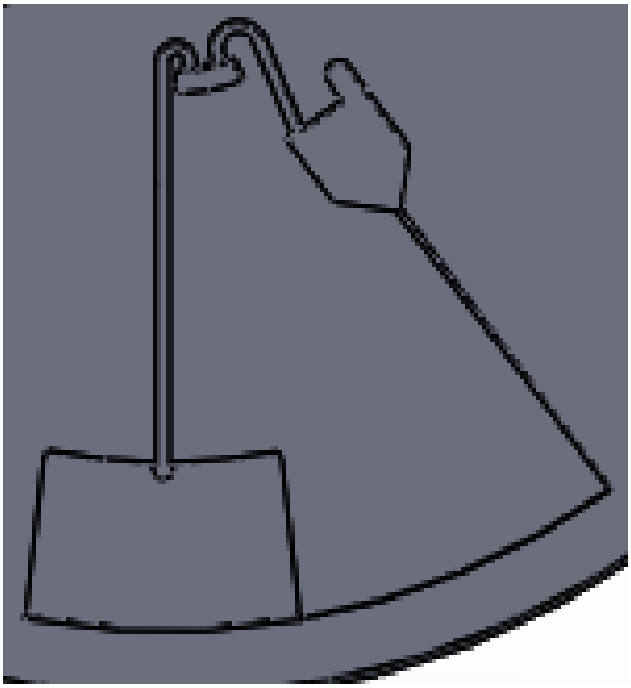


Figure 3.9: Trial4

3.4 Design Trial Results and Conclusions

Pre-screen experiment results

The figure below (3.10) shows the trials of the oil and design combinations. Bio-Rad oil was chosen owing to its compatibility with the selected Mastermix. The screening experiments showed significant changes in the number of droplets formed such that silicone oil had hardly few droplets whereas a significant number of droplets formed in Bio-Rad oil. It was suspected that the density difference between the mastermix and oil influenced heavily on whether the droplet channel should enter from the top or bottom. The lighter oils worked best when the channel entered from the bottom. Furthermore, the placement of the channel entrance at the center or the side depended on clogging of the channel. Centrally placed channels were impacted heavily by the centrifugal force which impeded the process of oil chipping off the droplets that formed. A side entrance enabled the oil to chip and carry the droplets that formed whereas in centrally placed units, the oil accumulated the droplets instead.

Oil	Trial	Compatibility with Emulsion
Silicon oil	MM flows from top on center (1)	No droplets formed
	MM flows from bottom on center (2)	
	Droplet Channel from bottom on the side(4)	
	Droplet Channel from top on side(3)	
Fluorinert	MM flows from top on center(1)	No Droplets
	MM flows from bottom on center	Droplets formed (Sparse)
	Droplet Channel from bottom on the side	Droplets formed (sparse)
	Droplet Channel from top on side	No droplets
Bio-Rad oil	MM flows from bottom on center	Droplets formed but clogged Channel entrance over time
	Droplet Channel from bottom on the side	Droplets formed

Figure 3.10: Design Trial Conclusions

To summarize the results, the combination of BioRad oil along with Design Trial 4 as shown in the image(3.9) was chosen using the pre-screen experiments. Furthermore, the final design parameters are tabulated below.

3.5 Droplet Formation Results from Design Trials

The droplets formed were not of quality as there was large size variation noticed. The mechanism for failure was analysed and the following two root causes were investigated:

- **Mechanism 1:** Spin Speed not sufficient to overcome air bubbles
- **Mechanism 2:** Chamber depth dimensions can contribute to volume loss of oil in turn causing coalescence of sample droplets

The image below depicts the first set of trial run results.

Design Parameter	Value	Observation
Channel width	$120\mu m$	Milling Limitation is $100\mu m$. Width determines droplet size
Channel Depth	$100\mu m$	Depth is critical to determine droplet size
Chamber width and length	$10mmx10mm$	Imaging lens is $10mm$ by $10mm$
Terrace width	$200\mu m$	Critical for shaping the droplets
Terrace Depth	$100\mu m$	Critical for shaping droplets
Oil Type	Bio-Rad	Contains surfactants, works best with the given mastermix
Oil:Mastermix	3 : 1	Most Optimal Ratio (from literature and initial screening experiments)

Table 3.2: Fixed List of Design Factors and Droplet formation recipe

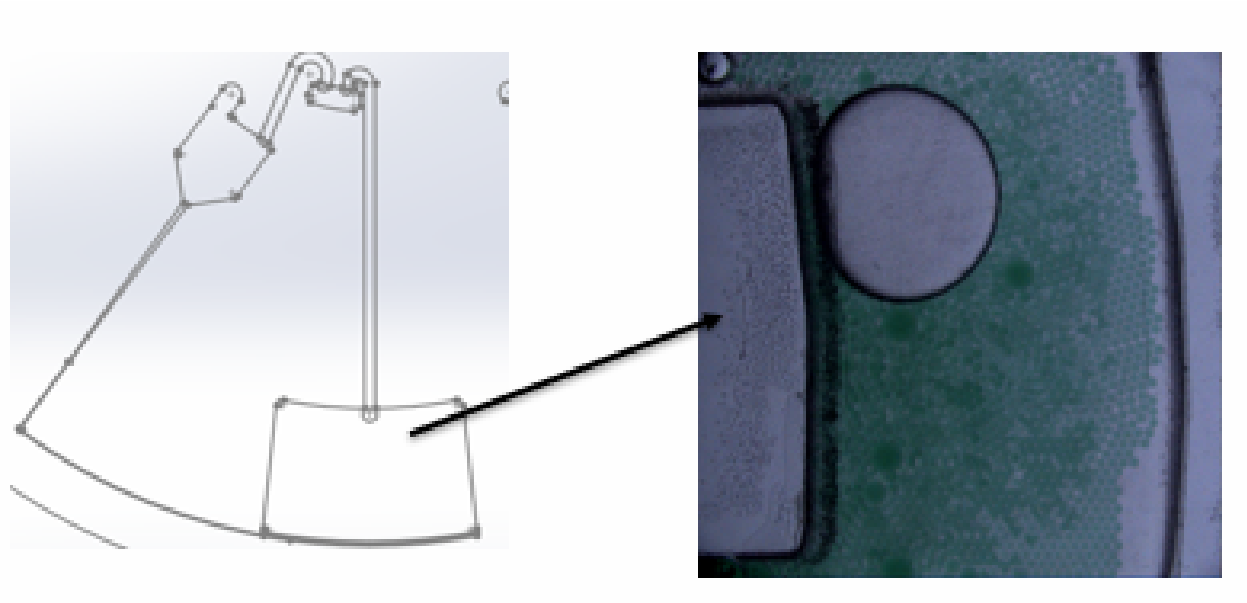


Figure 3.11: Results of droplet formation

3.5.1 Mechanism: Spin Speed

It is necessary to identify the optimum spin speed to generate the droplets. A low spin speed would not generate droplets whereas an extremely high spin speed would generate massive shear forces and separate the fluids into two separate phases instead of forming a stable emulsion. A speed to $1200rpm$ is required to generate droplets. Lower spin speeds also accentuate air bubble formation in the chamber and channels impeding fluid flow. The effect of spin speed was tested on the droplets after forming them. three different speeds were tested : $500rpm$, $1200rpm$ and $2500rpm$. The disc was spun for one hour for each of the trials. The results were analysed using ImageJ image analysis software. The depth of the chamber impeded the measurement capability of the droplet trials. The droplets tend to stack up and counting or measuring their diameter is tricky. The image below compared the droplets in these three speeds before and after spinning them. The number of droplets remained constant before and after the spinning experiment and amounted to on an average of 600 droplets on the visible layer of droplets. From the images its clear that spin speed

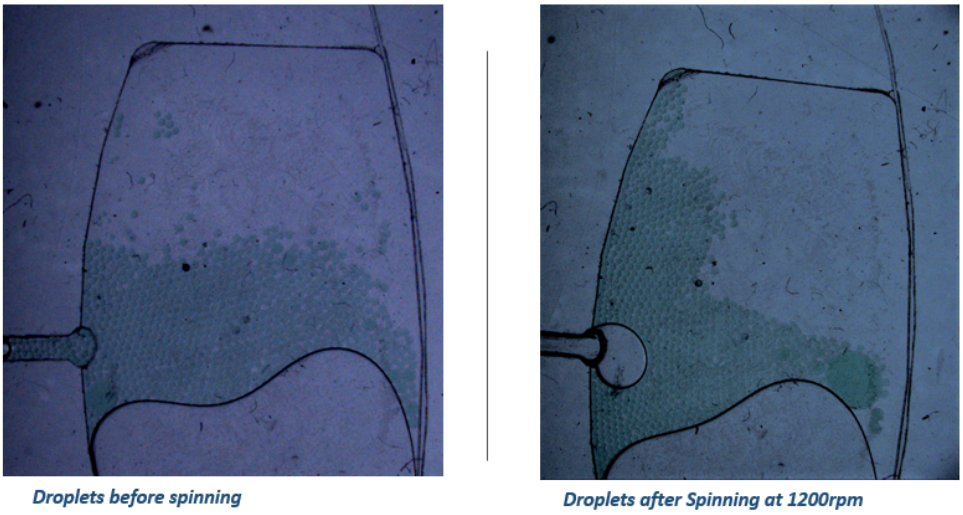


Figure 3.12: effect of Spin Speed at 1200rpm

may effect the formation of air bubbles but is not a critical factor influencing the droplet quality. A minimum speed of 500 rpm is required for the generation of droplets and a speed

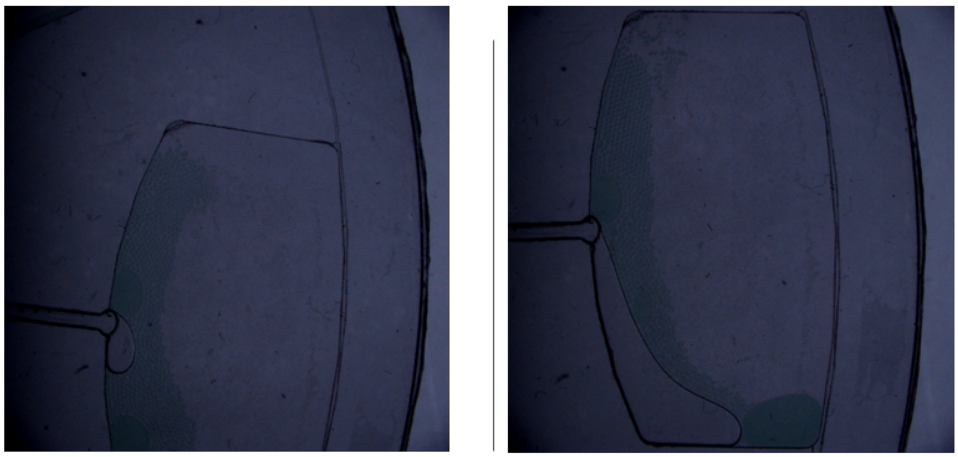
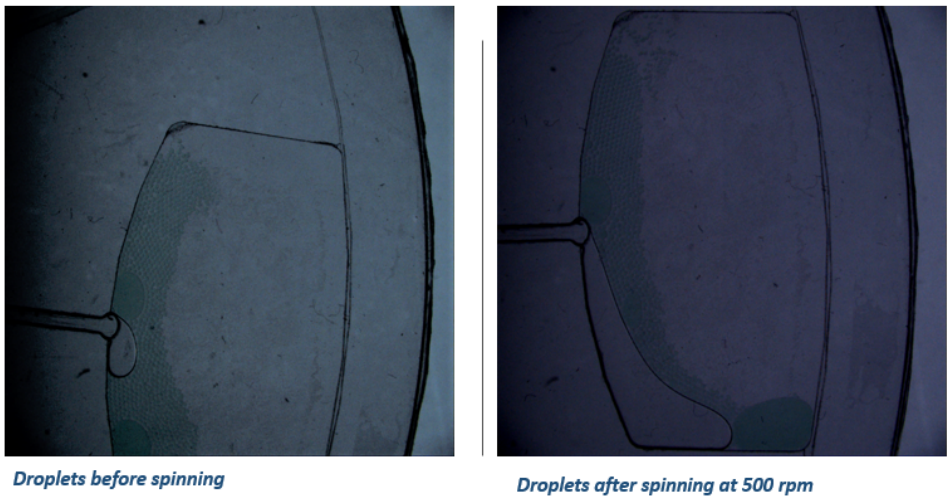


Figure 3.13: Effect of Spin Speed at 2500 rpm

Figure 3.14: Effect of Spin Speed at 500rpm



of upto 3000 rpm yields droplet formation beyond which the quality of droplets becomes unpredictable.

3.5.2 Mechanism: Chamber Depth

The key reason for investigating this mechanism is volume loss of oil due to leak or evaporation. As visible from the previous images, the droplet generation method yields a highly packed droplet formation unit. The coalescence of droplets occur when the oil separating two individual droplets is removed. That is, if the volume of oil in the chamber is lowered, the probability of coalescence would increase. Increasing the volume of oil for the same mastermix quantity would enable physical separation of droplets. this was investigated by forming droplets in chambers of different depths. Plots were generated to gauge the effect

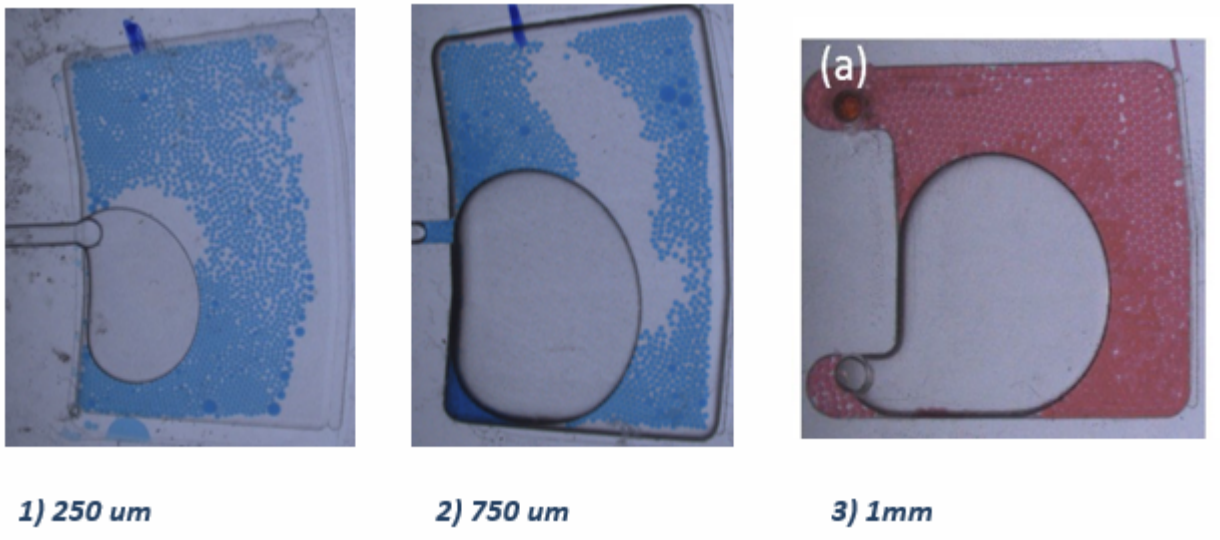
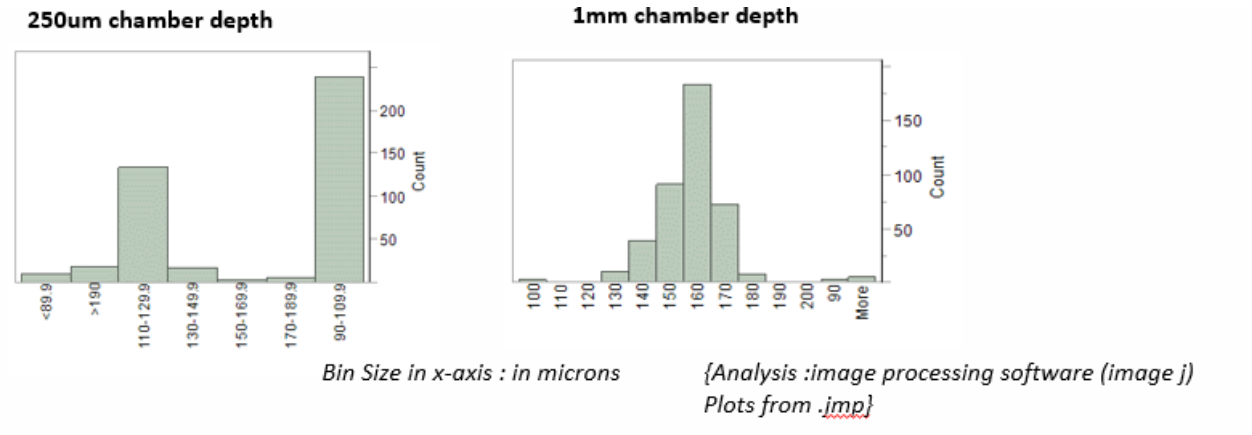


Figure 3.15: Effect of chamber depth

on droplet diameter for each of the cases. These plots are presented below. The chamber depth yielded a significant effect. Larger depths prevented coalescence but were difficult to analyse due to droplet stacking. Depths of $250\mu m$ had more coalescence whereas those with $1mm$ had considerably lower coalescence. This effect is specifically noticeable when PCR is

performed.

Figure 3.16: Histogram Plots of Droplet Size variation in Chamber Depth



3.5.3 Droplet PCR analysis

A fluorescence image of the PCR experiment of 30 cycles is presented below. The results from the experiment is analyzed to compute the effect on droplet size before and after temperature cycling.

With chambers of depth 250 microns, the oil tends to evaporate easily under PCR conditions. In deeper chambers the droplets survive the heating but there is considerable change in the droplet diameters. The droplet sizes were analysed and the results are plotted as below. Furthermore, the shape of the droplets also is disturbed under heating. A possible explanation for the same is also that the oil slips away easily from between the droplets under applied temperature. Smaller droplets therefore, get easily attached and a part of the bigger droplets yielding an overall larger size variation. A larger standard deviation is also noticed in the droplet size. Therefore, to conduct repeatable PCR experiments with droplets on the disc, more modifications with regards to chamber geometry would be required.

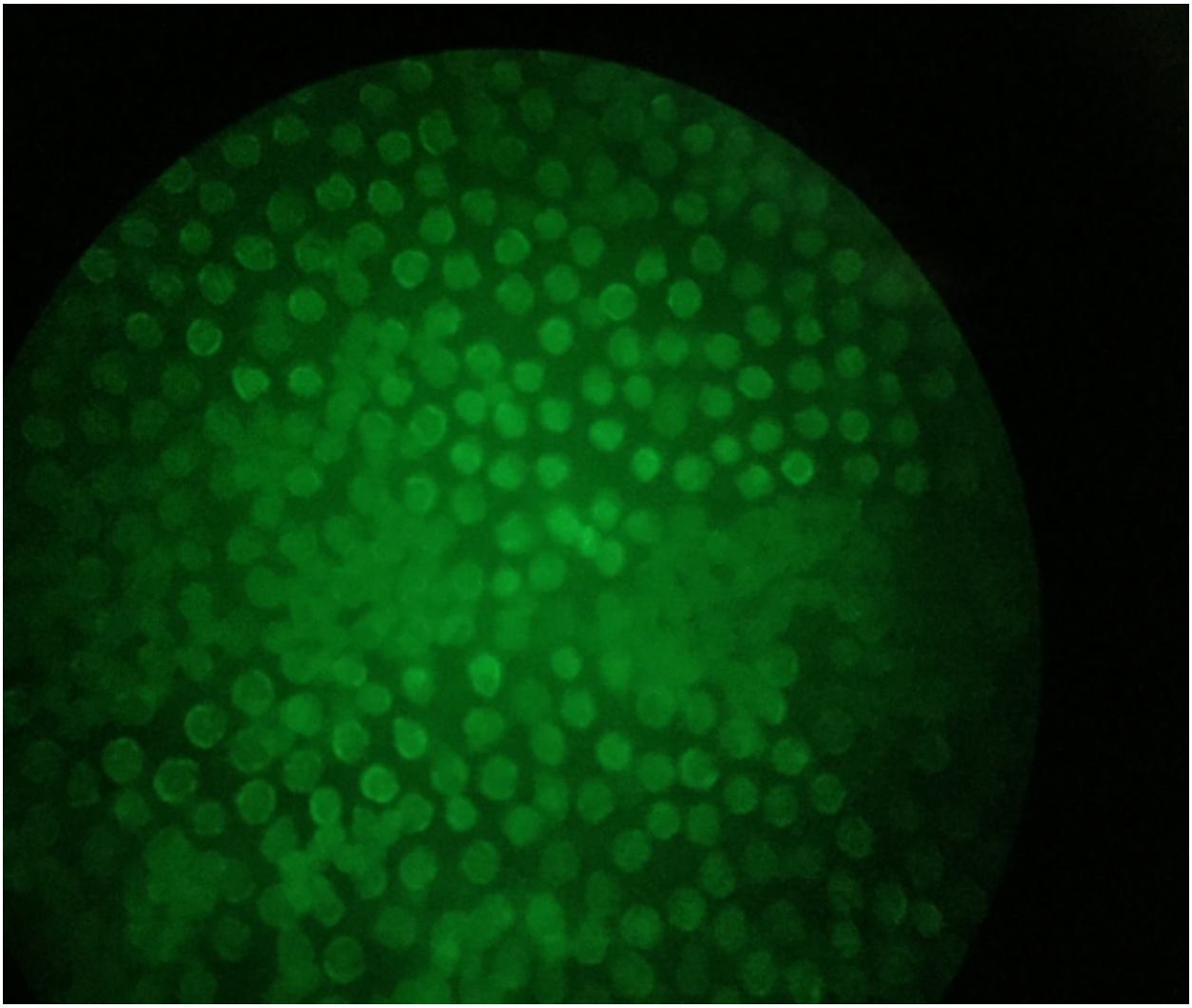


Figure 3.17: PCR Trial results

Process Conditions	**Mean Droplet Size**	**Standard Deviation**
$1mm$ Chamber before heating	$200.21\mu m$	14.36
$1mm$ Chamber after heating	$177.69\mu m$	25.88

Table 3.3: Droplet Size results from PCR Trial Experiments

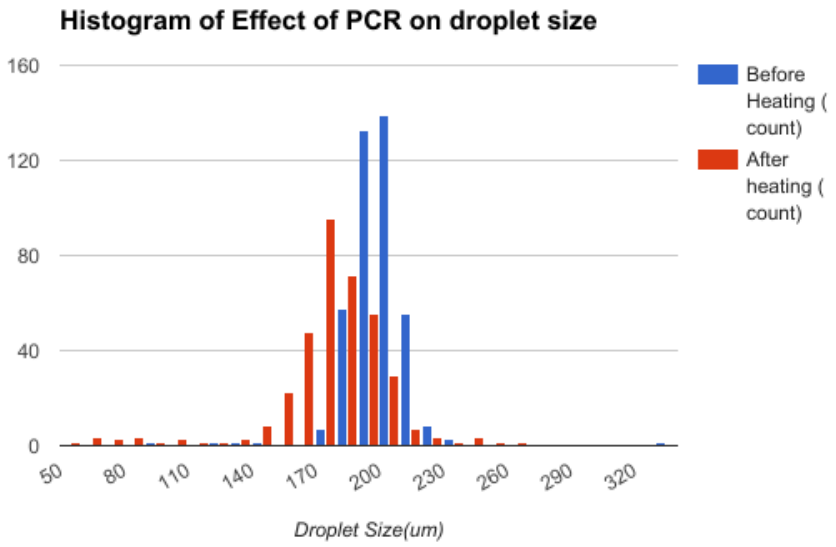


Figure 3.18: Histogram to compare the droplet quality under PCR conditions on a 1mm deep chamber

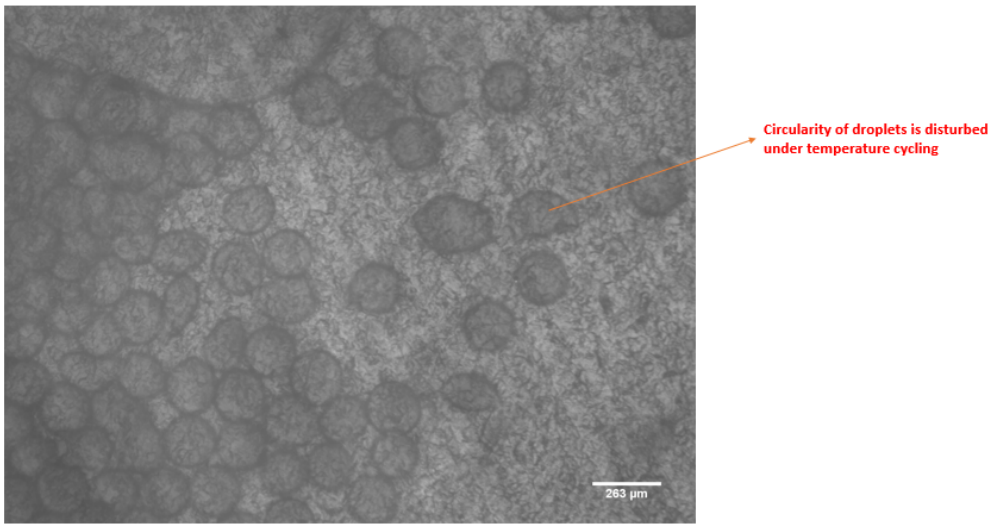


Figure 3.19: Change in droplet shape on heating in a 1mm chamber

Chapter 4

Generating Stable Agarose Droplets on a CD Microfluidic Platform

Droplet generation on the disc is primarily based on poisson statistics and does create many wasteful droplets or void droplets. Furthermore, polydispersity of droplets noticed in the previous section impedes the ability to amplify longer sample DNA amplicons required for complex genome assemblies. Lastly, use of fluorophores or microbeads for detection implies that PCR needs to be performed on a solid microbead. This causes stearic hindrances and affects reaction quality.[23] To enable efficient particle encapsulation in a droplet such as for single cell emulsion PCR, methods based on optics have been demonstrated like laser guided particle encapsulation. Another method is to use compliant gel particles which provides for a robust yet simple method for particle encapsulation in droplets. [3] Based along those lines, Leng et.al. [23] developed an agarose based droplet generation using co-flow on a Lab on Chip device. This work has attempted to create agarose based droplet generation on a CD to add to the flexibility of the CD based sample to answer system. This system attempts to combat the flaws in the poisson statistics based methods and to allow for more efficient emulsion based PCR. This section restricts itself to developing an efficient method

to generate the agarose droplets on the CD using step emulsification.

4.1 Materials, Methods and Experimental Set-up

Agarose(Ultra Low gelling Agarose from Sigma Aldrich) is chosen owing to its bio-compatibility and its unique sol-gel properties. The ultra-low gelling agarose melts at $56°C$ and solidifies at $4°C$. The agarose remains in a solid form until molten again. This enables conducting the PCR experiment within the liquid agarose droplets. After the completion of the reaction, the agarose droplets can be frozen to enable further analysis. This makes the agarose microbeads robust and with a longer shelf-life. The microfluidic disc is manufactured in a similar manner as that for mastermix in oil droplets as described in the previous section. The experimental set-up [Figure3.4] is kept constant while the operating conditions is modified to accomodate the change of materials. Before conducting design trials, viscosity data was obtained to gain a better understanding of the oil-agarose system.

4.1.1 Viscosity Plots

Viscosity variance with temperature was collected on the Bio-Rad oil and a 2% agarose solution with the help of UCI's Colloid Science Laboratory. The viscosity plots show that the viscosity of agarose remains higher than the viscosity of oil at elevated temperatures. This can be compared with a generic viscosity plot of water. This goes to prove that theoretically, an agarose-oil system would be more efficient in forming droplets.

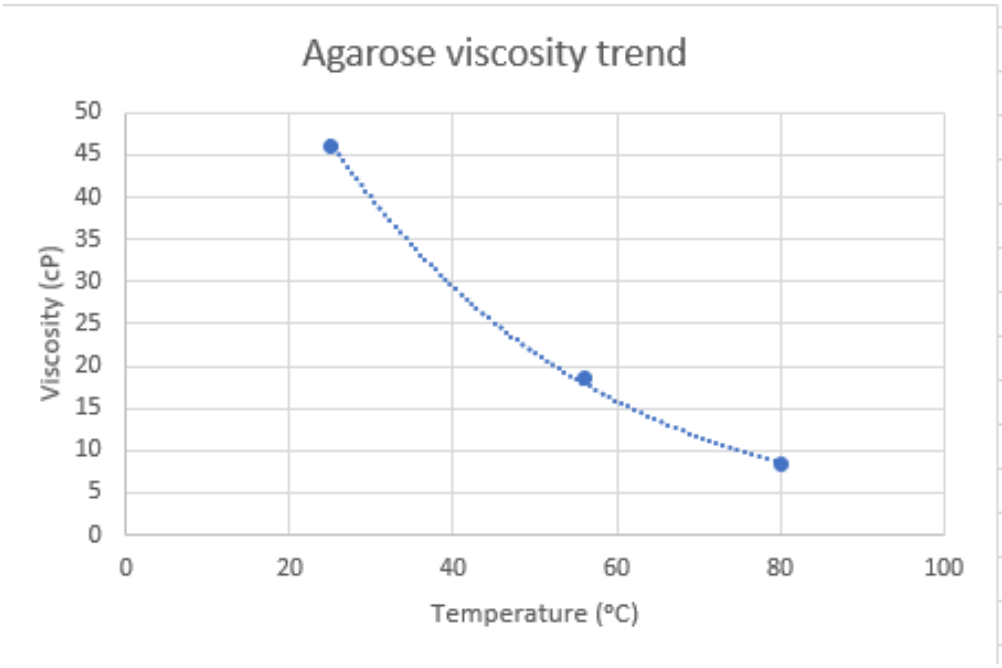


Figure 4.1: Agarose viscosity trend with respect to increasing temperature

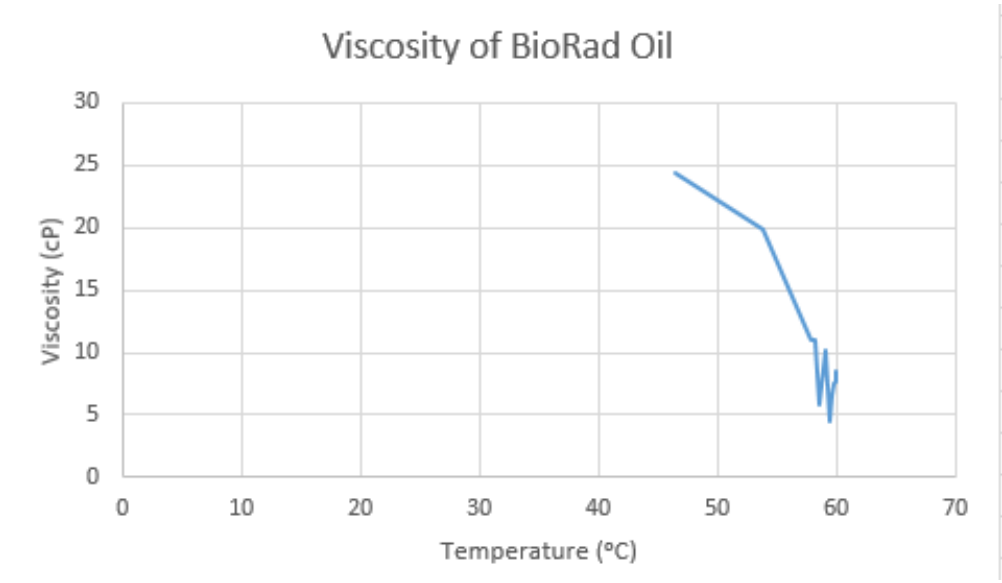


Figure 4.2: Bio-Rad oil viscosity trend with respect to increasing temperature

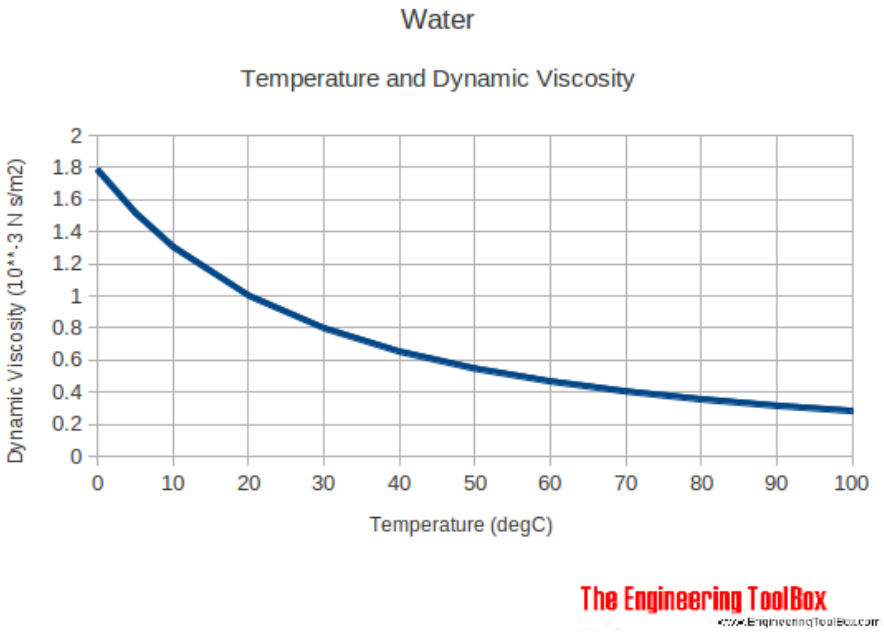


Figure 4.3: Generic viscosity plot for water with respect to increasing temperature(image source: engineering toolbox)

4.1.2 Method to generate agarose droplets

Two different agarose solutions were tried to establish a standard protocol to form agarose droplets. 0.02% agarose droplets were first formed by adding agarose to warm water [figure 4.4]. The droplets formed were stable and formed in the same operating conditions as that of water-oil systems(process parameters described in previous chapter). However, these droplets did not freeze easily and collapsed under PCR conditions. In order to enable formation of stable droplets with a long lifetime, the concentration of the agarose solution was increased to 2%. To form droplets using a solution of higher viscosity, a much higher spin speed was required. Furthermore, a lower packing of droplets within the chamber was required to avoid droplet coalescence during temperature cycling. Lastly, temperature was also applied during the process of emulsion formation. However, while this worked for the 0.02% solution, it promoted coalescence for the solution with increased viscosity. Therefore,

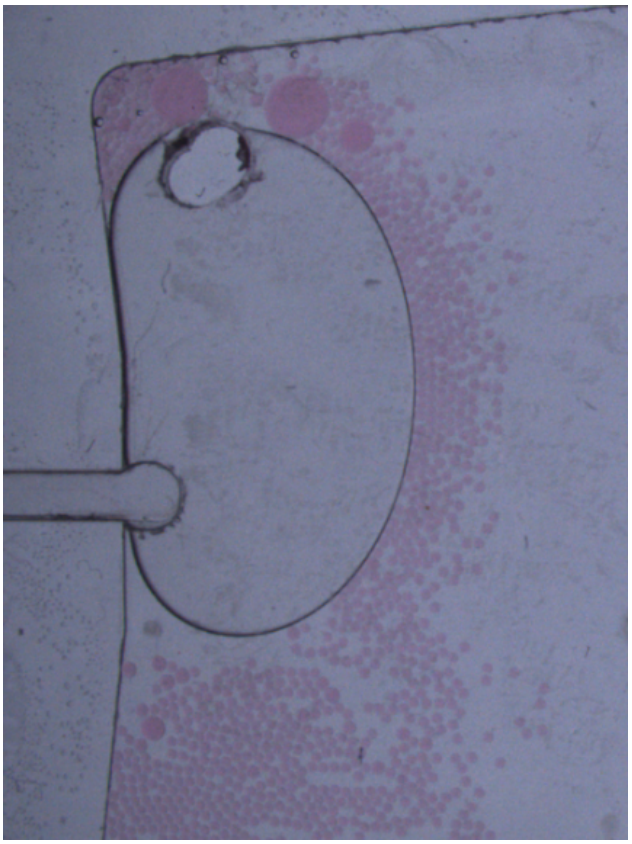


Figure 4.4: Droplets from dilute agarose solution

- 2% ultra Low gelling agarose in warm water
- Mixture was constantly heated and stirred for 20mins
- Oil was spun down the microfluidics disc under application of heat (this was done to avoid a thermal shock when the hot agarose contacts oil)
- Agarose added and disc spun down with no heat
- Droplets analyzed

Droplet Generation Parameter	Set values
Disc Design	*Same as MM in oil*
Agarose : Oil	*1:3*
Chamber Depth	*1.5mm (to reduce packing)*
Agarose solution	*2%*
Spin Speed	*2500rpm-5000rpm*
Spin Time	*3mins*

Figure 4.5: Process Parameters summary to generate agarose based droplets

4.2 Observation and Results:Particle Size analysis

The agarose-oil system produces much smaller and stable droplets as evident from the plot 4.6. This data was analysed using jmp statistical software. A table comparing the mean and standard deviation is drawn below. The standard deviation of the agarose system is much smaller showing a much lower variance in droplet size. This also indicates decreased coalescence in agarose droplets as compared to the aqueous droplets described in the previous

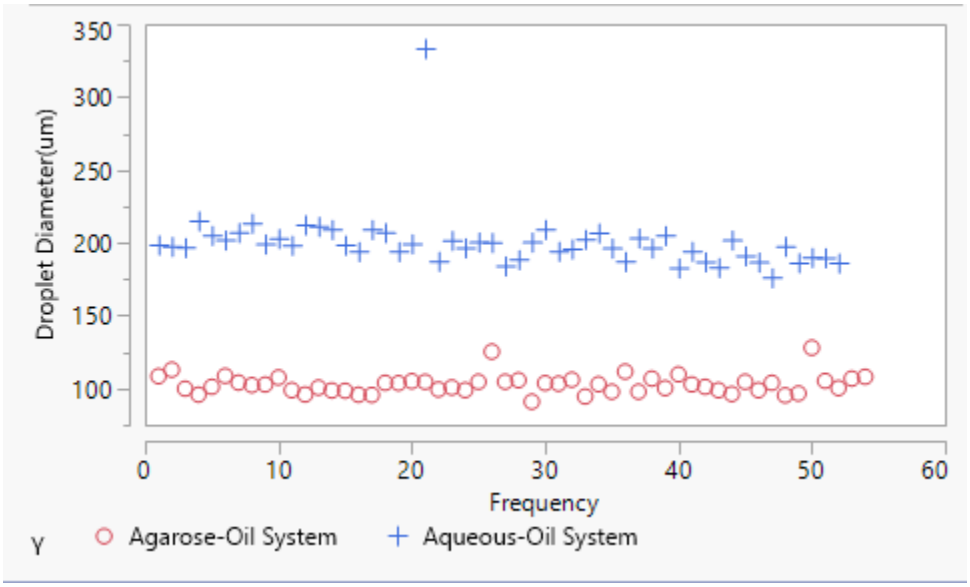


Figure 4.6: Plot Comparison of Aqeous-oil system and agarose oil system in terms of droplet size

Emulsion System type	**Mean Droplet Size**	**Standard Deviation**
Mastermix-Oil System(Aqueous-Oil)	$200.21\mu m$	14.36
2%Agarose-Oil System	$102.94\mu m$	6.49

Table 4.1: Droplet Size Comparison of Agarose-oil vs Aqueous-Oil Systems

chapter. Lastly, a proof of concept experiment was conducted to identify the potential of agarose based droplets for PCR. Pipette generated agarose droplets were placed on a cover slip which was sealed on to the disc chamber. These droplets were frozen to $4°C$ The PCR set-up was switched on to begin thermal cycling. The droplets could withstand the hot start temperature at $95°c$. Further design optimization is however required to implement PCR on agarose droplets generated using the CD platform.

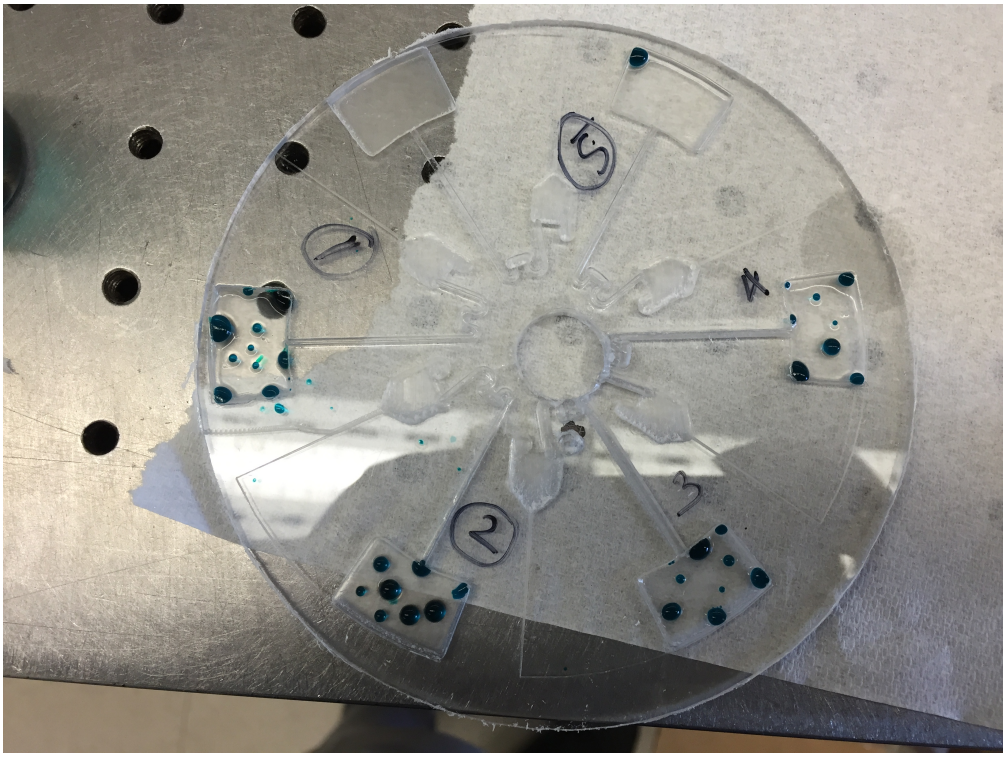


Figure 4.7: Pipette generated Agarose droplets

Chapter 5

Conclusion and Future Direction

CD Microfluidics is an exciting field for Extreme Point of Care research. This technology has a lot of potential in this large market for healthcare for developing nations. The integration of droplet microfluidics on the CD platform provides for a flexible and very powerful sample to answer system for the E-POC market. The possibility of integrating these two technologies have been successfully demonstrated bu to make the system commercially viable and to enable it to substitute a fully functional clinic is still a challenge.

5.1 Challenges and Future Work in Droplet Microfluidics on the CD Platform

The first and foremost challenge is to reduce the polydispersity of droplets especially in the aqueous-oil system. The large size variability is accentuated by the effect of temperature cycling making it an unstable system still for nucleic acid amplification. Although PCR has been demonstrated on this system, further work is required to improve the accuracy and reliability of the system. Furthermore, agarose based droplets seem like a good alternative

for aqueous droplets. However the merit of this system for temperature cycling is yet to be tested. Furthermore, in the agarose based system, another key factor was the design of the disc. The sharp droplet channel impedes flow of the viscous agarose solution. This mandates higher spin speeds. A modified channel design to ease fluid flow would improve droplet quality. Further experiments are required to understand the effect of surfactanats in combination with chamber depth to combat problems pertaining to coalescence. Lastly, the droplet quality would also be increased by decreasing droplet size to decrease packing density. This can be achieved with smaller droplet channels. Laser etching is a promising technology to make smaller features as opposed to CNC Milling. However, further tests are required to understand the impact of laser cut surface roughness on the hydrophobicity. Also, with decreasing droplet size, the effect of polydispersity is increased. this leads to coalescence of smaller droplets with larger ones to stabilize the system. However, droplet generation using step-emulsification provides a higher degree of control over the process as opposed two flowing phase systems. The simplicity of this system has yielded promising results in this integration step.

5.2 Future work on Extreme POC devices

To make this robust ideal E-POC device, further research is required on integration of different steps. Sample preparation step is the first challenge. Clinical settings are rarely available in resource-poor regions. Lack of trained manpower calls for a highly modular and user-friendly device. Therefore, an interface is required to ease the sample collection process and integrate it with the sample preparation step. Another area of research is to integrate reagent storage on disc to combat problems pertaining to refrigeration and storage owing to lack of power supply. In terms of the sample analysis and detection steps, the vital area of work is to integrate nucleic amplification accurately. The sensitivity of the device is crucial

to avoid wrongful detection of diseases and to combat the major problem of drug resistance that stems from this. Cost is a huge constraint and therefore another interesting outlook would be to look into cheaper manufacturing or on-site manufacturing. Low-cost materials for disposable devices is also a budding area of interest. Lastly, improving the capability of the device such that different assays can be run on the same modular, portable, CD platform is a key direction for E-POC devices.

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