ACTIVE DROPLET/BUBBLE GENERATION AND MEASUREMENT IN MICROFLUIDICS

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Abstract

This thesis deals with in-situ measurement and active control of droplet/bubble generation using ultrasonic.

The capability of injection molded PMMA device as a droplet generator was evaluated by characterizing the generated droplets under different sets of flow rates. A fully-automated video processing software application: Automatic Droplet Measurement (ADM) capable of in situ measurement has been built. This new software is faster than any existing available software and thus serves as an invaluable and efficient tool for droplet/bubble generation research. The ADM software has been publicly released for free.

Active control opens up a new space of degree of freedom to actuate and manipulate droplet/bubble sizes. A new method to control bubble generation using an ultrasonic transducer system has been developed. The gas–liquid oscillated by the system induces acoustic streaming, which increases the gas flow rate and bubble size significantly. The change in bubble size is repeatable with response in seconds. The same system has also been used to vibrate the oil-water interface for active droplet generation control. However, the effect is not as significant.
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<tr>
<td>AC</td>
<td>Alternating current</td>
</tr>
<tr>
<td>ADM</td>
<td>Automated Droplet Measurement</td>
</tr>
<tr>
<td>BAW</td>
<td>Bulk acoustic wave</td>
</tr>
<tr>
<td>BEO</td>
<td>Background extraction operation</td>
</tr>
<tr>
<td>BMG</td>
<td>Bulk metallic glass</td>
</tr>
<tr>
<td>BRO</td>
<td>Background removal operation</td>
</tr>
<tr>
<td>BTVS</td>
<td>Binary threshold value selection</td>
</tr>
<tr>
<td>CPU</td>
<td>Central processing unit</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>DC</td>
<td>Direct current</td>
</tr>
<tr>
<td>DI</td>
<td>Deionized</td>
</tr>
<tr>
<td>DMV</td>
<td>Droplet morphometry and velocimetry</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DOD</td>
<td>Drop on demand</td>
</tr>
<tr>
<td>DRIE</td>
<td>Deep reactive-ion etching</td>
</tr>
<tr>
<td>EMV</td>
<td>Electromechanical valves</td>
</tr>
<tr>
<td>ERF</td>
<td>Electrorheological fluids</td>
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<tr>
<td>EWOD</td>
<td>Electrowetting on dielectric</td>
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<tr>
<td>FIDT</td>
<td>Focused interdigital transducer</td>
</tr>
<tr>
<td>FPS</td>
<td>Frame per second</td>
</tr>
<tr>
<td>GERF</td>
<td>Giant electrorheological fluids</td>
</tr>
<tr>
<td>GUI</td>
<td>Graphical user interface</td>
</tr>
<tr>
<td>HDMS</td>
<td>Hexamethyldisilazane</td>
</tr>
<tr>
<td>IDT</td>
<td>Interdigital transducer</td>
</tr>
<tr>
<td>ITO</td>
<td>Indium tin oxide</td>
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<tr>
<td>KOH</td>
<td>Potassium hydroxide</td>
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<td>MATLAB</td>
<td>Matrix Laboratory</td>
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<tr>
<td>NOA81</td>
<td>Norland Optical Adhesive 81</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>OpenCV</td>
<td>Open Source Computer Vision</td>
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<tr>
<td>PBR</td>
<td>Preliminary background removal</td>
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<tr>
<td>PC</td>
<td>Personal computer</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<tr>
<td>PDMS</td>
<td>Polydimethylsiloxane</td>
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<tr>
<td>PFP</td>
<td>Perfluoroperhydrophenanthrene</td>
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<tr>
<td>PMMA</td>
<td>Poly(methyl methacrylate)</td>
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<tr>
<td>PTFE</td>
<td>Polytetrafluoroethylene</td>
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<tr>
<td>PVA</td>
<td>Polyvinyl alcohol</td>
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<tr>
<td>PWM</td>
<td>Pulse width modulation</td>
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<tr>
<td>RAM</td>
<td>Random-access memory</td>
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<tr>
<td>RC</td>
<td>Resistor–capacitor</td>
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<tr>
<td>RF</td>
<td>Radio frequency</td>
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<tr>
<td>RMS</td>
<td>Root mean square</td>
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<td>RS232</td>
<td>Recommended standard 232</td>
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<tr>
<td>SAW</td>
<td>Surface acoustic waves</td>
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<tr>
<td>SDK</td>
<td>Software development kit</td>
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<tr>
<td>SDOD</td>
<td>Steady drop on demand</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscope</td>
</tr>
<tr>
<td>UI</td>
<td>User interface</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>μ-PIV</td>
<td>Micro-particle image velocimetry</td>
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List of publications


Chapter 1: Introduction

1.1. Background

The rapid development of miniaturization in the past century yields numerous powerful products that are commonly used today but unthinkable in the past. For instance, smartphones which are used by 2 billion people around the world today are capable of fulfilling numerous functions that could only be done independently by several bulky devices half a century ago [1]. Miniaturization today may have fulfilled some of the challenges laid by Richard Feynman in his talk [2] given in 1959; however, there is still plenty of room at the bottom of the atomic scale.

Microfluidics is one of the miniaturization technologies that processes or manipulates small volume of fluid (10\(^{-9}\) to 10\(^{-18}\) liters) using microchannels (10\(^{-4}\) to 10\(^{-5}\) meters) [3]. The research on this field started about 40 years ago, with notable pioneering projects such as inkjet nozzles fabrication by IBM [4] and miniaturized gas chromatograph system by Stanford University [5]. The fabrication techniques of microfluidic device in the early days were largely adapted from microelectronics industry, which were well-established, precise and reliable albeit requiring expensive equipment. The major breakthrough to the microfluidics fabrication technique only took place about 14 years ago when rapid prototyping using polydimethylsiloxane (PDMS) was introduced [6]. This technique simplifies the fabrication process, reduces the fabrication time and thus lowers the cost required to fabricate microchannels or microsystems. Since then, this technique has been widely embraced and used; various new microfluidic prototypes have been created.
at a much greater pace and more ideas are tested in PDMS by the research community.

Today, the application of microfluidics could be found in several fields such as biology [7], (bio)chemistry [8], and the pharmaceutical industry [9]. In biology, microfluidics technology is used to fabricate devices for cell lysis [10], [11], cell sorting [12], [13], cell culture [14], electroporation [15], [16], flow cytometry [17], polymerase chain reaction (PCR) [18], [19], DNA sequencing [20] and immunoassay [21], [22]. In chemistry, miniaturized devices for electrophoresis [23], [24], chemical sorting [25], chromatography [26], chemical reactions [27] and enzymatic reaction [28] were designed. The pharmaceutical industry utilizes microfluidic devices such as microreactors to perform micro-syntheses [29], microsized fluorescence-activated cell-sorting devices to screen drug reactions [30], and micro droplet generator for molecular evolution [31].

In many microfluidics applications, droplets/bubbles are created by balancing the hydrodynamic forces in the microfluidic device. Droplets/bubbles are used in two phase micromixing [32], synthesis of material [33], examining chemical and physical interaction [34], [35] to biological studies for drug screening [36] and discovery [9]. Microfluidic devices of different designs including T-junction and co-flowing configurations have been widely used to generate droplets/bubbles [37], [38].

Typically, the size of droplet/bubble and generation frequency are controlled passively by varying the flow rate or the applied pressure [39]. Syringe pumps are usually employed to drive the flow with a controllable flow rate, while gravity-based
pressure unit or a pressure controller is used for pressure driven flows. The major drawback of passive control is the slow response time in the order of seconds or even minutes [40]. The long response time comes from the relatively large fluidic resistance of the tubing and the fluidic capacitance caused by the compressibility of the liquid or the channel material [41]. The only way to achieve a specific droplet size with preset flow rates or pressures is by adjusting the liquid properties and channel geometries.

Droplet/bubble generation can be controlled actively. The philosophy of active control is based on the possibilities of introducing “extra” energy into the system at the droplet/bubble formation point to drive the system out of its natural dynamic attractor/orbit. The energy can be introduced periodically or discretely, and following either synchronous or asynchronous actuation. The introduction of extra energy into the system using active control opens up an entirely new space of degrees of freedom to intelligently actuate and manipulate the sizes, structure or dispersion of droplets/bubbles.

A suitable measurement system is required to quantify the control and describe the quality of droplets/bubbles generated. Typically, the measurement parameters such as droplet/bubble size, volume and travelling speed are vital for quantification. The size distribution reflects the quality and the stability of the system. Micro-particle image velocimetry (μ-PIV) [42], laser setup [43] and video processing [44] can be used to measure the traveling droplets/bubbles in microfluidics. Among the measurement systems, video processing is widely used as it is label-free and able to extract many different parameters such as droplet/bubble area, production frequency and velocity.
1.2. Motivations and challenges

Bubble acoustic could be a suitable method to control bubble generation actively. It is low power and contactless with the liquid. In the research field, bubble acoustic is usually performed by trapping bubbles in blind side channels. The trapped semicylindrical air bubbles are difficult to form with a desired size as suitable wetting properties, blind side channel geometry, and initial flow conditions are required. Furthermore, the bubble size will change after a period of continuous oscillation.

Instead of trapping bubbles in blind side channels with challenging repeatability, it is proposed to create a forward moving gas-liquid interface with a pressure controller. The interface is then oscillated by an ultrasonic transducer to create an induced streaming around the interface. The induced streaming alters the behavior of bubble formation and the bubble size. This offers a new and novel way to control the generated bubble size while maintaining the fluid flow rate or pressure.

The only publicly available software for video processing, Droplet Morphometry and Velocimetry (DMV) [44], is not fully automated. Before analyzing the video frame by frame, the user needs to select a suitable scheme for background extraction operation (BEO) and select a threshold value for binary threshold value selection (BTVS). Moreover, the speed of the video processing using DMV is slow. These bottlenecks hamper the ability of using the software to reflect the result of the measurement quickly or let alone to perform in situ measurement on droplet/bubble. In situ measurement system can serve as an invaluable tool for droplet/bubble generation study as it allows users to implement immediate changes to rectify any abnormalities detected. For instance, leakage or the presence of
foreign particles in the microfluidic system will affect the flow behavior which in turn affects the size, speed and polydispersion of the generated droplets. This is also usually not apparent and obvious and can only be detected after a period of time or during routine checks. In certain cases, inconsistent results obtained after the evaluation entail repeated experiments which can be averted if in situ measurements are done promptly.

1.3. Research objectives and scope

This thesis has the following objectives and scopes:

1. To develop novel new and active control methods
   a. The setup will involve an ultrasonic transducer to allow acoustic control on the liquid-gas interface
   b. The setup will be applied on a closed-channel microfluidic system that generates bubble or droplet

2. To design and develop a new video processing software
   a. The software will be automated by having automation algorithm for background extraction and binary threshold selection.
   b. That software will enable in situ measurement of droplet/bubble
   c. The software will also help to evaluate the capability of the newly developed active method quickly and efficiently.

1.4. Thesis organization

This thesis consists of the following chapters:

Chapter 2 provides a comprehensive review of active control on droplet and bubble generation. Also, bubble acoustic and droplet/bubble measurement with video
processing in microfluidics are reviewed in the chapter. **Chapter 3** discusses the methods to fabricate a poly(methyl methacrylate) (PMMA) device for droplet generation. The characteristic of the droplet generation using the device is also presented in the chapter. **Chapter 4** presents the development of new video processing software for droplet measurements, the Automated Droplet Measurement (ADM) system, which allows integration with camera SDK for *in situ* measurement with high processing speed. The algorithms used to achieve full automation, i.e. object based BEO and automated BTVF, are also discussed in the chapter. **Chapter 5** introduces a new method to control the generated bubble size in a flow-focusing configuration using an ultrasonic transducer. The method is a low-cost and simple way to enhance the capability of a microfluidic bubble generator to produce a wide range of bubble size. **Chapter 6** discusses a method to maintain a gas-liquid interface at a junction with acceptable repeatability and maintainability using a pressure controller coupled to the gas. The effect of the interface vibrated by the ultrasonic transducer to the droplet generation and the main factor that contributes to the change in droplet size are discussed in the chapter. Finally, **chapter 7** summarizes the thesis with concluding remarks and recommendations for future works.
Chapter 2: Literature review

This chapter focuses on droplet and bubble generation in closed-channel microfluidic systems [45]. The length scales of the characteristic flow channels are smaller than one millimeter [41]. Only the flow of two phases are considered. The liquid phase of the droplets is the dispersed phase, and the surrounding fluid is the continuous phase. Active control of bubble generation & bubble acoustic is also presented briefly. The current droplet measurement software is also presented and discussed.

2.1. Droplet and bubble generation

The microfluidic channels for droplet/bubble generation are usually fabricated by sealing a flat substrate of fabricated microgrooves with another flat substrate [46]. This technique of fabrication proliferated after a quick and low cost rapid prototyping method using Polydimethylsiloxane (PDMS) [6] was introduced.

For droplet generation, the channel designs are generally categorized as cross-flowing and flow focusing. In cross-flowing design, microchannels are routed to make the dispersed phase to cross the flowing continuous phase at a junction, which can be in T-shape [47] or Y-shape [48]. The first planar microfluidic droplet generator reported by Thorsen et al. [49] is an example of a cross-flowing design. Depending on the geometry of the junction, the droplet formation using this design can either be unobstructed (much larger continuous phase channel width than the dispersed one [50], Figure 2-1) or obstructed (small enough continuous phase channel width such that the emerging droplet could obstruct most of the flow of continuous phase [51], Figure 2-2).
The use of flow focusing, was first introduced by Anna et al. [52]. In this design, the continuous phase is routed to contact the flowing dispersed phase symmetrically, as shown in Figure 2-3. The dispersed phase stream and its surrounding continuous fluid are then focused into an orifice downstream. At this instant, the squeezing pressure and viscous stress exerted from the surrounding phase makes the inner dispersed stream become thinner, followed by necking and eventually emerges as periodic droplets [53]. Depending on the flow condition and channels geometry, the droplet formation condition could be in dripping [54] or jetting regime [55].

Akin to droplet generation, both cross-flowing and flow focusing designs are also used for bubble generation. For cross-flowing design, gas from a side channel crosses the liquid phase at a T-shape junction [32]. The mechanism of bubble formation in obstructed cross flow is similar to droplet breakup in obstructed cross flow. For flow focusing design, the gas flowing at the center channel is pinched by
the liquid flowing symmetrically from two sides [56]. When the pinching process repeats periodically, bubbles with narrow size dispersion are formed.

Wetting properties of channel walls are important for stable generation of droplet in a microfluidic device [37]. In order to generate water droplets in oil, hydrophobic channel walls are necessary to ensure clean separation of water droplets at the formation site and to prevent the adherence of the droplets to the walls. Conversely, generation of oil droplets in water requires hydrophilic channel walls. For bubble generation, microfluidic devices with hydrophobic walls are usually used to generate bubbles in oil while hydrophilic walls for bubbles in water [57].

2.2. Active control of droplet generation

As discussed in the chapter 1, active control of bubble generation is done by introducing “extra” energy into the system at droplet formation site. The extra energy opens up new space of degrees of freedom to actuate and manipulate the sizes, structure or dispersion of droplets. Also, this type of control has the potential to achieve higher response time compared to the passive control. In following subsections, the active control methods are organized and discussed according to the type of external energy. The fundamental mechanism and the principal of each system with similar approaches will be discussed and compared.

2.2.1. Electrical control

Electric energy can be used to manipulate droplet generation. The combination of both mechanical and electric focusing forces at the microscale was initially proposed in a 3D configuration [58], [59]. That concept was subsequently incorporated in a 2D or planar microfluidic configuration by Anna et al. [52] and Link et al. [60], following the same path as the original 3D flow focusing [61], [62]. Combining forces
of completely different origins requires special consideration for the geometry and location of the electrodes. The electrodes should provide a main electric field component not only aligned with the mechanical force, but also having its maximum located around the same (focusing) region of the force [63]. The electrodes integrated into the system could either be in contact with the liquids or separated by a dielectric material to prevent electrode fouling.

Figure 2-4 Classification of electrical control approaches.
(a) Application of direct current on flow focusing configuration. The indium tin oxide (ITO) is patterned on a glass slide before being bonded to a PDMS device. [60]
(b) Flow focusing configuration with electrodes brought into contact with the fluids via insertion through PDMS. [64]
(c) Flow focusing device with EWOD control. [65]
(d) Flow focusing device fabricated using NOA 81 with EWOD control. An extra ITO electrode is placed on top of the channel to increase the effect of EWOD. [66]
(e) Flow focusing device fabricated with electrodes and its electrical connections. [43],[67]
*The dotted lines illustrate the changes after activation of the electrical control.

Electrical control of droplet generation can be categorized according to the type of current applied to the electrodes (Figure 2-4). For direct current (DC) control, the magnitude of voltage remains constant throughout the application of the current.
For alternating current (AC) control, the voltage fluctuates with a frequency that is different than that of droplet generation. For high-frequency AC control, the frequency of the control signal is much higher than that of droplet generation. Therefore, the droplet generation frequency determines the characteristic time of the system.

A. **Direct current**

Link et al. [60] employed a DC voltage to control the droplet formation in a microfluidic setup (Figure 2-4(a)). The planar flow focusing device had two electrodes made of indium tin oxide (ITO). The ITO electrodes were patterned on top of a glass slide before being bonded [68] to the PDMS part with microchannels. These electrodes had a direct contact with the liquids inside the channel. With an appropriate electrode design, the process of droplet formation changes upon application of a high voltage to a relatively conducting (or leaky dielectric [69]) liquid stream (Figure 2-4(a)). Water as a paradigmatic leaky dielectric has been extensively used in microfluidics. Water allows its free charges to quickly migrate in opposite directions under the applied electric field, until they hit the water–oil interface, where they accumulate [70]. Under a fixed set of flow rates, the droplet size decreases with increasing applied voltage, as a result of charge accumulation at the interfaces. The droplet volume can decrease by three orders of magnitude by increasing the voltage alone. In tangible terms, increasing the applied electric field $E \sim V/d$ demands an increase of interfacial area per unit volume $1/d$ to accommodate the extra induced charges, where $V$ is the applied voltage and $1/d$ is the characteristic surface curvature or the inverse of droplet size. This phenomenon can also be understood in terms of a simple capacitor model. The droplet interface
between the conductive water stream and the isolating oil stream acts as a capacitor, where the surface charge \( q \sim \varepsilon_0 E \) increases with the applied voltage. A larger surface area per unit volume, or overall surface curvature, leads to an increase in surface energy associated with surface tension. The normal stress balance at the water–oil interface is

\[
\gamma C = P + \left\{ \varepsilon_0 E_{0,n}^2 + (\varepsilon_i - \varepsilon_o) E_{i,n}^2 - \varepsilon_i E_i^2 \right\}, \tag{2-1}
\]

where \( \gamma \), \( \varepsilon_0 \), \( \varepsilon_i \), \( E_{0,n} \), \( E_{i,n} \) and \( E_i \) are the water–oil interfacial tension, electric permittivities of water and oil, normal components of the outer (oil) and inner (water) electric fields, and the tangential component of the electric field, respectively. And \( C \) is the local curvature of the interface. As long as the corresponding local increment in electric forces can be balanced by surface tension, the system remains balanced and stable. The local hydrostatic pressure \( p \) in the flow acts as a reservoir to guarantee the total surface stress balance at the water–oil interface as long as the electric stresses do not overcome the surface tension:

\[
\frac{\gamma}{d} \geq \varepsilon_0 E^2 \sim \varepsilon_0 \left( \frac{V}{d} \right)^2 \tag{2-2}
\]

However, as \( V \) increases, the electric forces increase faster (roughly as \( d^{-2} \)) than the surface tension (as \( d^{-2} \)), leading to instability, surface disruption, and ejection of the droplets. In other words, instability sets in when the local symmetry (balance) provided by eqn (2-1) is no longer possible. The increasing electric force decreases the break up time \( t_c \sim \left( \frac{\rho q^3}{\gamma} \right)^{1/2} \), thus forming smaller droplets under constant flow rates. If the applied voltage is too large, the interface anchored at the outlet and the
issuing droplet becomes highly charged at pinch off. The charge causes both of them to repel each other, promoting the droplet formation instability.

Electric control with direct current does not require moving parts. Besides, as charging and discharging of the interface can be quickly performed, the response time is reduced down to 10 μs [60]. However, electrode fouling is the main drawback of this technique. The upstream electrode is constantly in contact with the dispersed phase, while the downstream electrode only makes contact as the highly charged water droplets pass by. The electrode in contact with water is susceptible to fouling and could affect the reliability of the system [71]. Furthermore, the droplets produced by this approach are charged, and may not be suitable for sensitive chemical or biological samples.

The above concept was continued by Kim et al. without the ITO electrode [64]. The electrodes are in contact with the fluids through a metal wire directly inserted through the PDMS. Figure 2-4(b) shows this setup. Similar to Link’s result, the droplet size decreases with increasing applied voltage. However, instead of becoming an unstable stream when the applied voltage is too large, the dispersed stream of this setup forms a jet connecting the stream source to the ground electrode located downstream. Fine droplets with a diameter less than 1 μm were formed at a relatively low flow rate ratio between the dispersed and the continuous phase. The droplets are claimed to be formed from the tip of a Taylor cone, a cone-shaped interface with a 49.3° half-angle apex balanced by electrostatic force and surface tension [72]. However, the Taylor cone formed under this setup could only be kept stable for about a minute due to the pulsation exerted by the syringe pump run at a low flow rate.
B. Alternating current

In contrast to DC control, the electrodes of this approach are connected to an alternating current (AC) voltage. In terms of frequency, electric control with AC voltage can be further categorized into the two groups of low-frequency and high-frequency control. A frequency lower than the frequency of droplet generation is considered as low. A frequency above the droplet generation frequency is considered high. At low-frequency control, droplet generation is asynchronous and out of phase with the applied AC voltage, causing differences in the charge of individual droplets. The low-frequency approach was attempted by Kim to improve the repeatability of Taylor cone formation [64]. A pulse of 200 ms, with about 25 ms to ramp up/down, and an amplitude between 0 and 2000 V were applied to the electrodes. The Taylor cone is formed once per pulse.

Later, He et al. used a setup similar to Kim’s to investigate the response of droplet formation under low-frequency AC control [73]. In this work, the triangular AC signal had a frequency of 10 Hz and an amplitude of 2 kV. Interestingly, the change in the droplet size was independent of the polarity of the voltage. However, that change exhibited a hysteresis effect with the applied voltage. The droplet size decreased rapidly during the initial stage of the voltage ramp up. The droplet size then reached its minimum at about the middle of the ramp-up stage, before increasing slowly for the second half of the ramp-up stage. The droplet size continued to increase gradually for the whole ramp-down stage before restoring the original size near the end of the ramp-down stage. This phenomenon is related to the relaxation of flow rate oscillation, which can be explained by a model analogous to an RC electric circuit with varying resistance [73].
In high-frequency control, the average applied voltage during the generation of each droplet is approximately zero. In this approach, the droplet size is tuned by the frequency and the root-mean-square amplitude of the AC voltage. The electrowetting phenomenon can be observed in an electrolyte droplet on top of an electrode. The contact angle decreases upon application of a voltage across the electrolyte and the electrode. However, this phenomenon is typically only applicable up to about a hundred millivolts, before the occurrence of electrolysis [74]. The electrolysis problem is addressed by having a thin insulating film between the electrode and the electrolyte [75]. This configuration, also known as electrowetting on dielectric (EWOD), allows the application of a large voltage that further reduces the contact angle. Although both DC and AC can be used for EWOD, AC is generally superior as it reduces the contact angle hysteresis [76]. To our best knowledge, manipulation of droplet generation based on EWOD has been reported with AC voltage only. Malloggi et al. introduced the use of EWOD to control droplet generation on a flow focusing device [65]. The device consists of a PDMS part with flow focusing channels attached to an ITO glass precoated with a ~4 μm thick Teflon film (Figure 2-4(c)). An AC voltage (10 kHz, 0–170 V_{rms}) was applied on the aqueous phase through a thin wire while the ITO is grounded. A NaCl solution was used to enhance the conductivity of the aqueous phase. Both liquid phases were delivered individually into the device using hydrostatic heads.

The effect of electrowetting on the oil–water interface is indicated through the critical external water pressure $P_{W}^*$ required to sustain the interface at the flow focusing junction at a given external oil pressure $P_o$. The water pressure $P_{W}^*$ decreases with increasing voltage $V_{rms}$:
\[ P^*_W(V_{rms}, P_0) = P_L(V_{rms}) + \lambda P_0 \] (2-3)

where \( P_L(V_{rms}) \) is the maximum Laplace hydrostatic pressure needed to sustain the oil–water interface, and \( \lambda \) is a constant that depends on the hydrodynamic resistances of the channels. This model assumes that \( P^*_W \) needs to exceed the sum of \( P_L(U) \) and \( \lambda P_0 \) before droplets are generated.

In eqn (2-1), the local surface curvature is expressed as \( C = \left( \frac{1}{R_w} + \frac{1}{R_h} \right) \) where \( R_w \) and \( R_h \) are the two radii of curvature of the surface. In an analogous way to DC control, when an AC voltage is applied to the aqueous phase, the contact angle on the Teflon film decreases and \( R_h \) increases. In addition, increasing voltage \( V_{rms} \) may lead to an increase in electric stress on the oil–water interface. Both effects force a decrease in water pressure \( P^*_W \) to maintain the balance (eqn (2-3)). With constant \( R_w \) and \( \gamma, P_L(V_{rms}) \) decreases under the applied AC voltage. This model explains well the decrease in \( P^*_W \) with increasing \( V_{rms} \) in the experiment. Under a constant set of pressures, the droplet size increases with increasing voltage \([77]\). This effect is similar to an increase in the water flow rate owing to a smaller hydrostatic pressure at the outlet of the water channel. Since shear viscous forces dominate in the oil flow, the ratio between the viscous force and the electrostatic force on the water surface grows \([77]\), causing its destabilization. Mallogi et al. also reported the on-demand generation of droplets using the same setup by applying AC pulses. The droplet starts to form above a critical pulse width. The droplet volume could be tuned by the pulse width.

Active control using EWOD does not need a counter-electrode in contact with the fluids flowing at the downstream channel. Furthermore, the applied voltage is much
smaller than that needed for DC control. The lower voltage is gentler for the electrode that is in contact with the aqueous stream. However, the effectiveness of EWOD reduces dramatically if the interfacial tension is reduced by addition of surfactants such as Span 80 and Triton X-100 [77].

Gu et al. [78] reported a similar approach but using a much smaller orifice [79]. Instead of driving the oil flow using hydrostatic pressure, a syringe pump was used. A regime called tip-streaming was observed at low water pressure. The droplets formed in this regime had diameters between 1–2 μm and had a high spatial density. This phenomenon is similar to that caused by surfactant accumulation near the meniscus tip, as described by Anna and Mayer [80]. Gu et al. used a master parametric map [79] to show that upon increasing the applied voltage over a critical value from this regime, a “conical spray” regime occurred. In this regime, the droplets repel each other and spread out upon exiting the orifice. Interestingly, in this regime, the average droplet size increases with increasing voltage. The observed phenomenon might be related to the interplay between the extremely short relaxation timescale of the surfactant molecules at the interface tip and the applied frequency. However, in the dripping regime, the droplet size decreases with increasing voltage, as predicted by eqn (2-1).

In terms of device material, Gu et al. made the EWOD device using Norland Optical Adhesive (NOA) [66]. This material has a higher stiffness than PDMS, which enables the fabrication of channels with dimensions down to a few micrometers with a small aspect ratio. Furthermore, the material is compatible with a wider range of oils as compared to PDMS. Figure 2-4(d) shows that the effectiveness of electrowetting is further increased by having two ITO electrodes located on the top and the bottom of
the microchannel. Before using the device, the microchannels underwent salinization treatment to make them hydrophobic and more sensitive in electrowetting control. In this setup, both oil and water flow rates are driven by syringe pumps. Compared to PDMS devices, EWOD control on NOA devices is more stable. As two electrodes are located on the top and the bottom of the channel, the droplet size is more sensitive to the change in voltage.

EWOD-based control requires one of the electrodes to be in contact with the aqueous liquid. Thus, the electrode is still susceptible to fouling. The response of EWOD to AC voltage starts to deteriorate upon reaching a critical frequency at around 1 kHz for DI water with low conductivity [81]. The lower AC frequency may limit the production rate of monodisperse droplets. To address this problem, Tan et al. suggested a design different from the typical EWOD setup, where all the electrodes are not in contact with the liquids [43], [67]. This approach allows a higher frequency of up to 50 kHz to control the generation of low conductivity DI water droplets. Figure 2-4(e) illustrates the design of the planar flow focusing device. There are four electrodes, two upstream and two downstream, around the junction of the flow channel. The device is bonded to an ITO-coated glass. The ITO side is facing outward and is not in contact with the microfluidic channels. The electrodes were made by filling the microfluidic channels with indium using the technique called microsolidics [82]. The live terminal of the AC power supply is connected to the upstream electrode pair while the downstream electrode pair and the ITO film are grounded.

Under a constant set of flow rates, the droplet formation regime changes upon application of the AC voltage. Generally, the droplet size decreases with increasing
voltage. The droplet generation process also depends on the frequency and the conductivity of the aqueous phase. The initial dripping regime changes to the jetting regime when a high enough AC frequency is applied while using an aqueous phase with a low enough conductivity. The dripping regime remains unchanged at a low AC frequency and high conductivity. Interestingly, the droplet formation is unstable at the intermediate range of AC frequency and conductivity.

Experimental results suggest that the droplet size at the dripping regime was related to the voltage difference between the oil–water interface and the downstream electrodes. However, as the electrodes are not in contact with the liquids and measuring the voltage at the interface is difficult, the RMS voltage at the tip ($U_{\text{tip}}$) of the interface can be deduced using an RC circuit model. From that model, one obtains the equation,

$$U_{\text{tip}} = U_{\text{app}} \left(1 + \frac{1}{\frac{C_E}{C_i} + 2\pi j \left(\frac{f}{\kappa}\right) \left(\frac{C_E}{l}\right)}\right)^{-1},$$

where $U_{\text{app}}$ is the RMS voltage applied at the upstream pair electrodes, $C_E$ is the capacitance between the electrodes and the aqueous phase, $C_i$ is the capacitance between ITO and the aqueous phase, $j^2 = -1$, $f$ is the AC frequency, $\kappa$ is the conductivity of the aqueous phase, and $l$ is a geometrical constant. The results converge closely to a single curve on the graph of droplet diameter against $U_{\text{tip}}$.

Apart from the electrical model, Tan et al. [67] also proposed an electrohydrodynamic model that takes Maxwell stresses into consideration. The model relates the droplet size to an effective capillary number, $Ca_{\text{eff}}$:

19
\[ Ca_{eff} = \frac{Ca}{1 - B_e}, \]  

where \( Ca \) is the classical capillary number and \( B_e \) is the electric Bond number comparing the Laplace pressure with the Maxwell stress [81]. Although this model is able to describe the droplet generation process at a high frequency, it does not account for the conductivities of the fluid. Tan et al. later used this system to demonstrate the ability to swiftly modulate the frequency of droplet generation with a response time down to a few milliseconds [43]. By modulating the frequency in the range of 170–340 Hz, the system is fast enough to represent the main music tune of Ode to Joy in real time. The music tune is ‘played’ from the fluorescence signal tracing the frequency of droplet generation. The system is also able to represent the more challenging main music tune of the Flight of the Bumblebee with faster and larger frequency switching and larger error in the target frequency. Table 2.1 summarizes electrical control of droplet generation.
<table>
<thead>
<tr>
<th>Source</th>
<th>Type of current</th>
<th>Flow channel geometry$^a$</th>
<th>Fluids$^b$</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>[60]</td>
<td>Direct current</td>
<td>Flow focusing: C = oil</td>
<td>D = water</td>
<td>Voltage ≤ 800 V</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Orifice W = 30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[64]</td>
<td>Direct current, low-frequency pulse current</td>
<td>Flow focusing: C = mineral oil + 6% Span 80</td>
<td>D = distilled water</td>
<td>Voltage ≤ 2000 V</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H = 61.4, W = 100</td>
<td></td>
<td>Pulse frequency ≤ 5 Hz</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Orifice W = 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Downstream W = 150</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[73]</td>
<td>Low-frequency ac</td>
<td>Flow focusing: C = mineral oil (30 cP) + 6% Span 80</td>
<td>D = distilled water</td>
<td>Triangular ac voltage from 2 to 2 kV, 10 Hz</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H = 50, W = 92.7, 83.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Orifice W = 46.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Downstream W = 140</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[65]</td>
<td>High-frequency ac</td>
<td>Flow focusing: C = mineral oil (30 mPa s)</td>
<td>D = DI water + NaCl (1 S m$^{-1}$)</td>
<td>10 kHz AC source</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H = 35, 115, 160, 190</td>
<td></td>
<td>Voltage ≤ 170 V$_{ror}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>W = 390</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[77]</td>
<td>High-frequency ac</td>
<td>Flow focusing: C = mineral oil (30 mPa s)</td>
<td>D = DI water + NaCl (1 S m$^{-1}$)</td>
<td>10 kHz AC source</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H = 100, W = 390</td>
<td></td>
<td>Voltage ≤ 150 V$_{ror}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>For experiments with surfactants, either D + 0.1% Triton X-100 or C + 0.1% Span 80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[76]</td>
<td>High-frequency ac</td>
<td>Flow focusing: C = mineral oil + 3% Span 80</td>
<td>D = DI water + NaCl (0.5 S m$^{-1}$)</td>
<td>10 kHz AC source</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H = 50, W = 160</td>
<td></td>
<td>Voltage ≤ 55 V$_{ror}$</td>
</tr>
<tr>
<td>[79]</td>
<td>High-frequency ac</td>
<td>Flow focusing: C = mineral oil (30 mPa s) + 5% Span 80</td>
<td>D = DI water + NaCl (0.5-0.7 S m$^{-1}$)</td>
<td>10 kHz AC source</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H = 50, W = 200</td>
<td></td>
<td>Voltage ≤ 70 V$_{ror}$</td>
</tr>
<tr>
<td>[66]</td>
<td>High-frequency ac</td>
<td>Flow focusing: C = mineral oil (30 mPa s) + 5% Span 80</td>
<td>D = DI water + NaCl (0.5-0.7 S m$^{-1}$)</td>
<td>10 kHz AC source</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H = 10, W = 160</td>
<td></td>
<td>Voltage ≤ 160 V$_{ror}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Orifice W = 50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[43], [67]</td>
<td>High-frequency ac</td>
<td>Flow focusing: C = mineral oil (30 mPa s) + 5% Span 80</td>
<td>D = DI water + NaCl (3 × 10$^{-7}$-0.3 S m$^{-1}$)</td>
<td>5-50 kHz AC source</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H = 35, W = 100</td>
<td></td>
<td>Voltage ≤ 1000 V$_{ror}$</td>
</tr>
</tbody>
</table>

$^a$ $H$ – height in μm; $W$ – width in μm. $^b$ C – continuous phase; D – dispersed phase.
2.2.2. Thermal control

Figure 2-5 Two approaches for thermal control.
(a) Flow focusing geometry with integrated heater and temperature sensor at the orifice of droplet formation. [83]
(b) A heat exchanger regulates the temperature of the upstream part at a temperature ranging from 0 to 90 °C. The other one keeps the downstream channel at room temperature. Teflon separates the heat exchangers as an insulator. [84]
(c) Location of the spot applied with localized heat by laser and the stream contour of the Marangoni flow formed around the droplet. [85]
(d) Droplet produced on demand by creating cavitation bubbles using a high intensity laser. [86]
*The dotted lines illustrate the changes after activation of the thermal control.

Thermal control of droplet generation can be categorized into two approaches according to the way the heat is introduced. The first approach utilizes resistive heating at the junction where the droplets are formed. The temperature is controlled by the applied current and feedback from a temperature sensor. The second approach utilizes a focused laser beam to achieve
localized heating. Figure 2-5 gives an overview of both approaches. Each approach has its own merits and limitations which are discussed as follows.

The use of resistive heating to manipulate droplet size was first introduced by Nguyen's group [83], [87]. An integrated microheater and a temperature sensor were used to control both the droplet generation regimes and the droplet size in a microfluidic flow-focusing configuration (Figure 2-5(a)) [83]. The microheater and temperature sensor made of platinum were fabricated using a standard lift-off process. The heater and sensor were insulated from the fluids by a thin PDMS layer to avoid charging of the fluids or possible electrolysis.

Thermal control is based on the temperature dependency of the fluid properties, mainly the viscosity and interfacial tension. For most fluids, viscosity and interfacial tension decrease with increasing temperature. This change is reflected in the change in capillary number $Ca$. In passive droplet generation, the capillary number is often used to characterize both the diameter and the formation regime of the droplets. The dependency of the normalized droplet diameters for the set of fluids used was expressed as [83]

$$D^*(\Delta T) \propto \frac{\gamma^* T}{\eta^* T} = e^{0.02\Delta T},$$

(2-6)

where $D^*$, $\gamma^*$ and $\eta^*$ are the diameter, interfacial tension and viscosity normalized by values at a reference temperature. The diameters of the droplets increase by about 2 times with temperature increasing from 25 °C to 70 °C. The above exponential scaling is only valid for the given fluid system, and does not fully describe the complex nature of thermal control of droplet generation. Factors such as the Marangoni effect due to the spatial temperature distribution, surfactant
concentration, flow rate ratios, accuracies in both viscosity and surface tension measurements and droplet formation regimes were neglected and not considered in the analysis. This approach offers the compactness and portability required by different lab-on-a-chip applications due to the small footprint. However, the fabrication of the microheater and temperature sensor is complicated. The alignment of the microheater and temperature sensor at the desired location adds further complexity towards the implementation of this concept.

The same group extended the work further to investigate the effect of nanoparticles in different microfluidic geometries and channel heights [88]–[90]. Spherical TiO$_2$ nanoparticles of 15 nm diameter were added to water leading to a reduction of the interfacial tension between water and oil. The reduction was attributed to the Brownian motion of the nanoparticles. The viscosity and interfacial tension decrease almost linearly with increasing temperature. A microfluidic T-junction device with a channel height of about 90 μm, a side channel width of 50 μm and a main channel width of 150 μm was used to investigate the temperature dependence of the droplets formed using both DI water and DI water with nanoparticles as the dispersed phase fluids. Experimental results showed that for the case of DI water, the size of the droplet increases slightly from about 180 to 190 μm. However, with the nanoparticles, the size of the droplet increases from about 220 to 260 μm. The small sensitivity to temperature is caused by the squeezing regime of droplet formation [32]. The capillary numbers Ca are $2.8 \times 10^{-3}$ and $4.1 \times 10^{-3}$. With these small Ca numbers, pressure forces dominate over the viscous forces and the size of the droplets depends mainly on the applied flow rate ratio. Therefore, the size of the droplets correlates weakly with the temperature. However, if the channel height is reduced from 90 to 30 μm, the temperature dependence of the droplet size
increases due to the larger temperature gradient [89]. Interestingly, the temperature dependence of nanofluid droplets changes when a flow focusing configuration is used [90]. In this configuration, both DI water and nanofluid exhibit similar characteristics in droplet formation at different temperatures. However, the transition between the droplet formation regimes differs for both fluids. This difference is caused by the complex behavior of nanofluids, which introduces factors such as interfacial slip and Brownian motion.

Heating the entire microfluidic device also provides thermal control. Stan et al. [84] placed a microfluidic flow focusing droplet generator on a pair of heat exchangers. One heat exchanger regulates the temperature of the upstream part from 0 to 90 °C, while the other exchanger keeps the downstream channel at room temperature (Figure 2-5(b)). Three continuous liquid phases were used, namely Light mineral oil (Sigma Aldrich 330779), Dynalene SF and perfluoroperhydrophenanthrene (PFP, Alfa Aesar L17370). Dynalene SF showed the largest increase in water droplet size. At 70 °C, the droplet volume is 100 times smaller than the original volume at 10 °C. The use of the heat exchanger extended the range of temperature below room temperature and avoided the integration of micrometer and temperature sensors.

Localized heating at the droplet generation site can be achieved precisely with a focused laser beam. Heating using a laser is more flexible as the position of the focused laser spot can be adjusted easily. Baroud et al. used a focused laser beam to achieve active control of droplet generation [85]. An argon-ion laser with a wavelength of 514 nm was focused slightly downstream of a cross junction (Figure 2-5(c)). During the droplet generation process, the advancing oil–water interface was blocked at the laser spot (80 mW beam power, 5.2 μm beam waist). The interface continues to advance downstream as the viscous stress grows to overcome
the blocking force. As the droplet formation process is delayed under constant flow rates, the droplet size is about 2 times bigger than that produced without heating with a laser.

Tracing the microparticles indicated that a flow is generated around the laser spot. The dashed lines in Figure 2-5(c) represent the flow pattern schematically. This flow was induced by the Marangoni effect. Localized heating led to a large temperature gradient and consequently a surface tension gradient at the liquid interface [85]. The induced flow is large enough to prevent the interface from advancing downstream. The blocking time is directly proportional to the beam power. For instance, with a continuous phase flow rate of $Q_c = 6 \mu L h^{-1}$ and a dispersed phase flow rate of $Q_d = 0.18 \mu L h^{-1}$, the blocking time increases from 0.8 to 1.6 s with the laser power increasing from 60 to 80 mW. The focused laser beam has also been applied to merge two droplets, to fuse droplets at formation, to split droplets, and to direct the droplets [91].

Extending the concept of laser heating, Park et al. applied a pulse laser on a stable water–oil interface [86] to generate water droplets on demand with a valve (Figure 2-5(d)). The laser beam caused localized heating with boiling and rapidly created a cavitation bubble. The bubble injects a droplet from the interface into the surrounding oil phase. The flow rates used in that work ranged from 12 to 190 mL h$^{-1}$ for water and 0.2 to 6.5 mL h$^{-1}$ for oil. A laser with a high pulse frequency would allow a high droplet generation rate of up to 10 000 droplets per second. However, as the process is temperature dependent and repeated laser pulses steadily increase the temperature of the system, the system temperature has to be regulated. Table 2.2 summarizes thermal control of droplet generation.
Table 2.2 Thermal control

<table>
<thead>
<tr>
<th>Source</th>
<th>Heating element</th>
<th>Flow channel geometry</th>
<th>Fluids</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>[83]</td>
<td>Platinum microheater</td>
<td>Flow focusing: $H = 70$</td>
<td>$C = \text{MO} + 2%_{\text{w/w}} \text{Span 80}$</td>
<td>$T = 25 - 75$; $Q_1 = 600$, $Q_2 = 50$; $+200%$ from $d = 39.6$; $Q_0 = 100$, $+180%$ from $d = 30.9$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Orifice $W = 45$</td>
<td>$D = \text{DI water} + 0.05%_{\text{w/w}} \text{FD}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Channel $W = 200$</td>
<td>$D_1 = \text{DI water} + 0.05%_{\text{w/w}} \text{FD}$</td>
<td></td>
</tr>
<tr>
<td>[88]</td>
<td>Platinum microheater</td>
<td>Flow focusing: $H = 90$</td>
<td>$C = \text{MO} + 2%_{\text{w/w}} \text{Span 80}$</td>
<td>$T = 25 - 56$; $Q_0 = 300$, $Q_1 = 60$; $+5.6%$ from $d = 180$ with $D_3$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Main $W = 150$, Side $W = 50$</td>
<td>$D_2 = \text{DI water} + 0.05%_{\text{w/w}} \text{FD}$</td>
<td>$+16.1%$ from $d = 224$ with $D_3$</td>
</tr>
<tr>
<td>[90]</td>
<td>Platinum microheater</td>
<td>Flow focusing: $H = 30$</td>
<td>$C = \text{MO} + 2%_{\text{w/w}} \text{Span 80}$</td>
<td>$T = 25 - 45$; $Q_0 = 60$, $Q_1 = 5$;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Orifice $W = 45$</td>
<td>$D_3 = \text{DI water} + 0.05%_{\text{w/w}} \text{FD}$</td>
<td>$+97%$ from $d = 61.8$ with $D_3$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Channel $W = 200$</td>
<td>$D_4 = \text{DI water} + 0.05%_{\text{w/w}} \text{FD}$</td>
<td>$+153%$ from $d = 49.3$ with $D_3$</td>
</tr>
<tr>
<td>[89]</td>
<td>Platinum microheater</td>
<td>Flow focusing: $H = 300 &amp; 30$</td>
<td>$C = \text{MO} + 2%_{\text{w/w}} \text{Span 80}$</td>
<td>$T = 25 - 39$;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Main $W = 100$</td>
<td>$D_1 = \text{DI water} + 0.05%_{\text{w/w}} \text{FD}$</td>
<td>$H = 300$, $Q_0 = 120$, $Q_1 = 66$; $+12%$ from $d = 334$ with $D_3$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Side $W = 50$</td>
<td>$D_2 = \text{DI water} + 0.05%_{\text{w/w}} \text{FD}$</td>
<td>$H = 30$, $Q_0 = 12$, $Q_1 = 6$;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Orifice $W = 40$</td>
<td>$D_3 = \text{DI water} + 0.1%_{\text{w/w}} \text{cylindrical TIO}<em>2 + 0.05%</em>{\text{w/w}} \text{FD}$</td>
<td>$+53%$ from $d = 85$ with $D_3$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Channel $W = 200$</td>
<td>$D_4 = \text{DI water} + 0.1%_{\text{w/w}} \text{FD}$</td>
<td>$+12%$ from $d = 64$ with $D_3$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Main $W = 100$</td>
<td>$D_5 = \text{DI water} + 0.1%_{\text{w/w}} \text{cylindrical TIO}<em>2 + 0.05%</em>{\text{w/w}} \text{FD}$</td>
<td>$-15%$ from $d = 106$ with $D_3$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$H = 25 - 40$</td>
<td>$T = 10 - 70$</td>
<td></td>
</tr>
<tr>
<td>[84]</td>
<td>Heat exchanger</td>
<td>Flow focusing: $H = 125$</td>
<td>$C = \text{Dymalene SF}$</td>
<td>Total flow rate $= 5.3$ mL h$^{-1}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Orifice $W = 40$</td>
<td>$D = \text{water}$</td>
<td>Volume increases 100 times of the original volume</td>
</tr>
<tr>
<td>[85]</td>
<td>Argon-ion laser</td>
<td>Channel $W = 200$</td>
<td>$C = \text{hexadecane} + 2%_{\text{w/w}} \text{Span 80}$</td>
<td>Laser power $= 80$ mW</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Main $W = 200$</td>
<td>$D = \text{water} + 0.1%_{\text{w/w}} \text{fluorescein}$</td>
<td>$Q_0 = 54$, $Q_1 = 4.8$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$H = 30$</td>
<td>$D_1 = \text{DI water} + 0.05%_{\text{w/w}} \text{FD}$</td>
<td>Droplet volume increases about 2 times of the original volume</td>
</tr>
<tr>
<td>[86]</td>
<td>Q-switched Nd:YVO$_4$ pulsed laser</td>
<td>Side $W = 125$</td>
<td>$C = \text{Corn oil}$</td>
<td>$Q_0 = 200-6500$, $Q_1 = 12.000-198.000$;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Parallel channels with orifice: $H = 100$, $W = 100$</td>
<td>$D = \text{Phosphate-buffered saline buffer}$</td>
<td>Droplet on demand generation with frequency up to 10 000</td>
</tr>
</tbody>
</table>

$\text{a} \ C$ - continuous phase; $D$ - dispersed phase; $\text{MO}$ - mineral oil ($\text{Sigma M5904}$); $\text{FD}$ - fluorescent dye ($\text{Sigma F6377}$); $H$ - height in $\text{m}$; $W$ - width in $\text{m}$; $d$ - diameter in $\text{m}$; $T$ - temperature in $^\circ\text{C}$; $Q_1$ - continuous phase flow rate in $\text{mL h}^{-1}$; $Q_0$ - dispersed phase flow rate in $\text{mL h}^{-1}$; $\text{spherical TIO}_2$ has a size of 15 nm; $\text{cylindrical TIO}_2$ has a size of $10 \times 40$ nm.
2.2.3. Magnetic control

Figure 2-6 Classification of the approaches for magnetic control.
T-junction device with ferrofluid and magnet [92] at (a) the upstream position and (b) the downstream position. Droplet generation in a uniform magnetic field with a C-shaped electromagnet, [93], [94] (c) direction of the magnetic field, \( H \), and (d) direction of the magnetic field when the polarity is inversed. Flow focusing junction configuration under homogeneous magnetic field [93] in the direction (e) parallel to the downstream channel and in (f) inversed polarity from (e). Creation of homogeneous magnetic field using permanent magnets [95]. The in-plane magnetic field could either be (g) parallel to the downstream channel or (h) perpendicular to the downstream channel. The gray arrows show the direction of the magnetic field, \( H \). (i) Ferrofluid droplet generation under out-of-plane, homogeneous magnetic field, \( H \) [96].

* The dotted lines illustrate the changes after activation of the magnetic control.

A liquid may exhibit a bulk dynamic response to a magnetic field. Magnetism allows contactless actuation in microfluidics such as pumping, mixing, trapping, separation and detection [97], [98]. Magnetism has also been adopted to control the generation
[92], transport [99], [100], splitting [101], morphology manipulation [102] and positioning [103] of droplets. Here, we focus only on droplet generation. Magnetic fluids such as ferrofluids are liquids with suspended magnetic particles. A magnetic fluid may serve as either the dispersed phase or the continuous phase [104]. The interparticle magnetic energy in a ferrofluid is weak because of the small particle size of less than 10 nm and the surfactant coating. Thus, thermal energy can overcome magnetic potential and evenly distributes the magnetic particles by Brownian motion [105]. A ferrofluid can be treated as a continuum [104]. Ferrofluids are superparamagnetic and can be magnetized without magnetic memory. The nanoparticles in a ferrofluid become non-magnetic once the external magnetic field is removed [97]. A ferrofluid can either be oil-based or water-based. Ferrofluids have been used in microfluidics to manipulate droplet generation magnetically (Figure 2-6).

Droplet generation with a water-based ferrofluid was first reported by Nguyen's group [92]. The ferrofluid is injected through a T-junction as the dispersed phase (Figure 2-6(a and b)). A small circular neodymium iron boron (NdFeB) magnet (3 mm in diameter, 2 mm thick) was placed under the device to influence the generation process. The strength and direction of the magnetic field were adjusted by the position of the permanent magnet. Under a set of constant flow rates, the droplet size changes when the magnet is placed upstream (Figure 2-6(a)) or downstream (Figure 2-6(b)) of the T-junction. The induced magnetic force may delay or accelerate the generation process, leading to different droplet sizes. The magnetic effect decreases with increasing total flow rate due to the larger pressure and viscous forces.
Magnetic control is applicable to an existing droplet generating device with a slight modification to place the permanent magnet. Since the ferrofluid is the dispersed phase, the droplet may not be suitable for samples that are not compatible with the ferrofluid. Furthermore, the magnetic gradient is fixed due to the placement of a relatively large permanent magnet. Thus, the strength of the induced force may not be enough to control the generation process, especially at a high generation frequency. This is why the initial setup [92] was improved in Nguyen’s later works with a C-shaped electromagnet [93], [94] (Figure 2-6(c and d)). This setup allows the convenient adjustment of the magnetic field strength by varying the applied current to the electromagnet. The droplet size decreases with increasing magnetic field applied along the main channel of the T-junction (Figure 2-6(c)). Interestingly, this behavior does not change with the opposite field polarity, Figure 2-6(d)) [106].

The ferrofluid aligns itself with the magnetic field and is stretched along the field direction leading to faster breakup and smaller droplets. This phenomenon is independent of the polarity of the magnetic field, producing similar results for both polarities.

When the same uniform magnetic field is applied to a flow-focusing configuration, the droplet size increases with increasing magnetic flux density. The magnetic field is parallel to the main channel (Figure 2-6(e)). The ferrofluid stream is stretched along the magnetic field direction. Secondary flows are formed within the ferrofluid tip resulting from the alignment of the nanoparticles to the magnetic field [93]. These two factors slow down the breakup process and consequently increase the droplet size. Similar to the T-junction, this effect is independent of the polarity of the magnetic field (Figure 2-6(f)).
The experimental results obtained from the flow-focusing configuration were consistent with the simulation based on the finite volume method and the particle level set method. The model utilized the augmented Navier–Stokes equation coupled with interfacial force and magnetic force [94]:

$$\frac{\partial}{\partial t} (\rho \vec{u}) + \nabla \cdot (\rho \vec{u} \vec{u}) = -\nabla p + \nabla \cdot \left[ \eta \left( \nabla \vec{u} + \nabla \vec{u}^T \right) \right] - \gamma \kappa \vec{n} \cdot D(\phi) - \frac{1}{2} \mu_0 |\vec{H}|^2 \nabla \chi_m, \quad (2-7)$$

where the third term on the right side is the interfacial force and the last term is the magnetic force. Consistent with the experiments, the simulation showed that in the presence of a magnetic field, the tip of the ferrofluid is stretched forward and the formation time is longer, promoting the formation of larger droplets.

Wu et al. further investigated the generation process of ferrofluid droplets by applying an in-plane, homogeneous magnetic field on a flow-focusing configuration [95]. The results are consistent with previous studies with the applied magnetic field parallel to the downstream channel (Figure 2-6(g)). Furthermore, the droplet size also increases with the magnetic field perpendicular to the downstream channel (Figure 2-6(h)). Although both field directions increase the droplet size, the droplet generation mechanisms are different. The emerging droplet is stretched sidewards when the magnetic field is applied perpendicular to the downstream channel. As a result, the forming droplet delays the breakup leading to a larger size.

The generation process of a ferrofluid droplet also responds to an out-of-plane, homogeneous magnetic field [96]. Lee et al. investigated the generation of ferrofluid droplets in a T-junction configuration (Figure 2-6(i)). The generation frequency decreases as the applied field strength increases. At the first observation, the results seem to violate mass conservation as the measured droplet diameter is proportional
to the generation frequency. However, a more detailed analysis shows that the results still follow mass conservation. The droplet deforms from a hemisphere to a hemi-ellipsoid after being exposed to the magnetic field, making the hemi-ellipsoidal droplet diameter appears smaller as viewed from the top, while the droplet volume actually increases. To our best knowledge, magnetic control of droplet generation has been applied only to ferrofluid as the dispersed phase. Magnetic control of droplet generation is still emerging with much room for new discoveries. For instance, the generation of aqueous droplets in an oil-based ferrofluid has not been explored. Table 2.3 summarizes magnetic control of droplet generation.

### Table 2.3 Magnetic control

<table>
<thead>
<tr>
<th>Source</th>
<th>Magnetic field</th>
<th>Flow channel geometry&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Fluids&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>[92]</td>
<td>Non-uniform</td>
<td>T-junction: H = 100, Main W = 300, side W = 50</td>
<td>C = silicone oil (100 cSt s) D = Ferrotech EMG 807</td>
<td>B &lt; 28 mT</td>
</tr>
<tr>
<td>[93], [94]</td>
<td>Uniform, in-plane, parallel to downstream channel</td>
<td>H = 100, W = 100, Orifice W = 45, Flow focusing</td>
<td>C = silicone oil (100 cSt s) D = Ferrotech EMG 807</td>
<td>B &lt; 45 mT</td>
</tr>
<tr>
<td>[106]</td>
<td>Uniform, in-plane, parallel to downstream channel</td>
<td>H = 100, W = 100, Orifice W = 50, T-junction: H = 100</td>
<td>C = silicone oil (100 cSt s) D = Ferrotech EMG 807</td>
<td>B &lt; 45 mT</td>
</tr>
<tr>
<td>[95]</td>
<td>Uniform, in-plane, parallel and perpendicular to downstream channel</td>
<td>Flow focusing</td>
<td>C = mineral oil + 4% Span 20 D = Ferrotech EMG 807</td>
<td>B &lt; 32 mT</td>
</tr>
<tr>
<td>[96]</td>
<td>Uniform, out-of-plane</td>
<td>H = 400, W = 400, T-junction: H = 90</td>
<td>C = tripropylene glycol diacylate (TPGDA) D = Ferrotech EMG 705</td>
<td>B &lt; 9 mT</td>
</tr>
</tbody>
</table>

<sup>a</sup> H = height in μm; W = width in μm. <sup>b</sup> C = continuous phase; D = dispersed phase.

#### 2.2.4. Mechanical control

Mechanical control of droplet generation involves physical deformation of the liquid interface using hydraulic, pneumatic or piezoelectric actuation. Piezoelectric actuation will be discussed separately from the former two methods as it has unique characteristics caused by the much faster response time.
A. **Hydraulic/pneumatic control**

Figure 2-7 Classification of the hydraulic/pneumatic control approaches.

(a) T-junction with actuation channel [107]; (b) side view along the actuation channel (dashed line). (c) Actuation channels connected to 10 valves which are used to chop the dispersed stream into individual droplets [108]. (d) Horizontal moving valve connected to actuation channel [109]. (e) On demand droplet generation using rounded profile T-junction channel and membrane valve [110]; (f) multiples of T-junctions to produce droplets with distinct composition [111]; (g) stiff NOA81 device with an active connector [112]; (h) polycarbonate slabs with a nitrile membrane [113]. (i) A valve is actuated by expanding the actuation channel pneumatically. This reduces the channel size around the orifice and obstructs the dispersed phase stream, resulting in a reduction of the droplet size [114]; (j) integrated adjustable orifice plate at the dispersed phase channel [115]. *The dotted lines illustrate the changes after activation of the hydraulic/pneumatic control.*
Hydraulic and pneumatic actuations are usually executed by valves integrated into the microfluidic devices. Generally, the valves are made of the same elastic device material such as PDMS. The valves are actuated pneumatically using compressed air or hydraulically by applying pressure to the liquid filled valve chamber. If the characteristic time $t_v$ of the actuation has the same order of magnitude as the droplet generation time $t_d$, the actuation is considered as dynamic. The droplet generation is controlled by the transient effect of the valves. In contrast, the actuation is considered static if $t_v \gg t_d$. Figure 2-7 summarizes the different approaches for active control with hydraulic and pneumatic actuation.

**Perturbation method**

The frequency of droplet generation $f = t_d^{-1}$ can be influenced by imposing physical perturbation near the generation site. Willaime et al. [107] created perturbation with a vibrating mechanical valve integrated into the side channel of the T-junction. The device was fabricated using multilayer soft lithography (Figure 2-7(a and b)). The top and bottom layers bear the actuation channel and the flow channel, respectively. A PDMS membrane is sandwiched between the two layers and serves as the valve membrane. Water in the valve channels deflects the membrane when a pressure is applied.

At a fixed set of flow rates without perturbation, the T-junction generates droplets steadily with a fundamental frequency, $f = f_0$. The generation frequency, $f$, changed after introducing the system perturbation. The droplets could either be generated irregularly with high variation in droplet sizes (quasi-periodic regime), or regularly with $f$ correlated with the perturbation frequency, $f_p$ (synchronized regime). In the synchronized regime, $f_p$ is near $f_0$ or a multiple of $f_0$. For instance, if the pressure
amplitude ranges from 0.7 to 1.4 bar, with \( Q_d = 18 \) and \( Q_c = 240 \, \mu\text{L h}^{-1} \), the synchronized regime appears at a frequency ratio of:

\[
f_p/f_0 \approx 1, \frac{3}{2}, 2, 3, 4, 5, \quad (2-8)
\]

where the generation frequency, \( f \), for each case is synchronized with \( f_p \) in the following manner

\[
\frac{f}{f_p} = 1, \frac{2}{3}, \frac{1}{2}, \frac{1}{3}, \frac{1}{4}, \frac{1}{5}, \quad (2-9)
\]

respectively.

By choosing a suitable pressure amplitude and plotting the susceptibility \( S = \frac{\partial f_0}{\partial Q_d} \) against \( f_0/f_p \), an optimized flow rate \( Q_d \) can be found within a wide range of \( f_p \) producing \( f/f_p = 1 \). Obtaining a wide range of generation frequencies \( f \) and droplet sizes at a fixed set of flow rates is important to achieve active control. For instance, with \( P = 1.7 \) bar and fixed flow rates of \( Q_d = 6 \) and \( Q_c = 240 \, \mu\text{L h}^{-1} \) the system can deliver a generation frequency \( f \) from 2 to 30 Hz and a corresponding droplet volume from 100 to 1000 pL. The advantage of this design is the small perturbation needed to influence the droplet generation frequency. However, the system needs to be fully characterized to determine the optimized flow rates and pressure amplitude for synchronized droplet generation. The applicable flow rates are limited as for most of the flow rates the perturbation induces the quasi-periodic regime that produces droplets with an irregular period and size.
**Chopping method**

Instead of using a vibrating valve to perturb the system, Lee's group incorporated a train of ten similar valves as choppers to cut a pre-focused dispersed stream into droplets [108]. The narrow stream was formed by the continuous phase at the cross junction under stable conditions all the way to the outlet. The valves are located near the outlet of the channel and connected to a common actuation channel (Figure 2-7(c)). Compared to the perturbation method, the chopping method presents a direct way to produce droplets at a desired frequency. This method also allows droplets to be formed at a high flow rate ratio between the dispersed and the continuous phase, because the chopping force from the valves is significantly higher than the viscous force imposed by the continuous phase. However, the generation frequency of this technique is limited by the actuation frequency of the valve. Furthermore, the droplet size disparity could increase as some of the droplets generated upstream could be chopped again by the valves downstream.

Instead of incorporating vertically moving valves that require a multilayer fabrication process, Lee's group later used a pair of horizontally moving valves [109] which were easier to fabricate. The design requires the fabrication of only one layer of PDMS as the actuation channels are now in the same plane as the droplet channel (Figure 2-7(d)). The horizontally actuating valves deform the main channel, which chops the narrow stream and forms the droplets. The drawback of this design is the relatively low generation frequency of only up to 17.4 Hz.

**Flow manipulation**

When the amplitude of the dynamically actuated valve is large enough to induce intermittent flow on the dispersed phase, droplets can be generated on demand. Lin
et al. incorporated a membrane valve on top of a T-junction channel [110] (Figure 2-7(e)). The droplets are generated at the T-junction by driving the dispersed phase to the main channel and the continuous phase to the side channel with individually controlled pressures. The valve is placed at the main channel, upstream of the junction. The cross section of the flow channel is rounded to facilitate total blockage of the flow when the valve is closed.

Steady on demand generation of droplets (Steady Drop on Demand, SDOD) was performed by full control of the instantaneous flow rates, generally of the dispersed phase. Lin et al. [110] activated the valve periodically while maintaining constant pressures on the dispersed and continuous phases. The resulting droplet volume \( V \) increases with increasing opening duration \( T_0 \):

\[ V = kT_0^a, \]  

where \( k \) and \( a \) are constants that depend on the flow conditions, such as the driving pressures \( P_d \) and \( P_c \) of the dispersed and continuous phases. Since the flow is transient right after opening the valve, it results in \( a > 1 \). Transient flow occurs as a consequence of the abrupt negative pressure gradient created during the retreat of the membrane. The transient effect was found to decrease, yielding \( a \approx 1 \) with increasing ratio of \( P_d/P_c \). The relationship (eqn (2-10)) is empirical. Hydrodynamic factors such as flow impedance and viscosity of fluids were not considered.

SDOD provides extra degrees of freedom for the controllability of the droplet generation process. The approach is able to produce a selected droplet volume within a large range. Also, the timing of droplet generation is fully controllable [110]. However, the frequency of droplet generation is limited by the time taken to open
or close the valves. As a result, the frequency of droplet generation is limited to below 20 Hz. The system by Lin et al. requires a round profile to allow sufficient flow blockage when the valve is fully expanded. The channel cannot be fabricated using SU-8 resin unless it is post-processed using a photo-polymerization technique [115].

Lee et al. proposed a predictive model for Drop on Demand (DOD) generation [116]. The dispersed phase was introduced from the side channel and the continuous phase through the central one. Using the analogy to an electronic circuit, a fluidic circuit model was formulated. The flow rate of the dispensed phase \( Q_d \) was determined by solving the equations with Kirchhoff’s circuit laws. Finally, the theoretical droplet volume can be simply calculated as \( V_{theo} = Q_d t \), where \( t \) is the activation time. Instead of the applied pressure at the inlet, Zeng et al. [111] used negative pressure at the outlet for the DOD concept. The droplet size measured in the area increased linearly with \( T_0 \) in the pressure range from −28.6 to −44.4 kPa. With the pressure as a single driving parameter, the system is easy to operate. Multiple T-junctions with individually controllable pneumatic valves allow for the generation of droplets from different sources (Figure 2-7(f)).

Ochs et al. designed pincer microvalves that do not only allow DOD generation but also the control of the dispersed phase flow rate [117]. Leung et al., on the other hand, embedded a series of microvalves that make up a programmable droplet-based microfluidic device for sample compartmentalization [118]. The response of DOD can be further improved using devices made of a stiff material [112]. However, integrated membrane valves can only be fabricated in devices made of elastomer such as PDMS. Galas et al. solved this problem by decoupling the device
design from the flow controlling system [112]. An active connector made of PDMS with an integrated membrane valve controls the flow of the dispersed phase (Figure 2-7(g)). The rest of the device is made of a hard material (NOA81).

Churski et al. reported a similar approach using a stiff device and an external valve module [113], [119]. The module is modified from an off-the-shelf electromagnetic valve (EMV) to reduce the flow through the valve and lessen the build-up of pressure at the downstream [119]. It can also be modified from an EMV using a slab of polycarbonate and a nitrile membrane [113]. In order to further shorten the opening and closing time of the valve, a pair of EMVs is also used for each inlet (Figure 2-7(h)) [113]. EMV_{high} is connected to a compressed air source while EMV_{low} is connected to an atmospheric pressure or vacuum source. As a result, the minimum time $T_0$ required for on demand generation is reduced to 25 ms, about half the time needed by a setup using one EMV only. In their later work, Churski et al. also provided practical guidelines for the use of low-cost EMVs to generate nanoliter droplets on demand [120].

**Geometry tuning**

Lee's group used a statically actuated valve for active droplet generation [121]. The horizontally moving valve is located along one side of the downstream channel. The activated valve reduces the width of the channel leading to a narrower stream of the pre-focused dispersed phase and consequently smaller droplets. The same valve type was used in a flow-focusing device without the chopping mechanism [114] (Figure 2-7(i)). The valves allow the size of the orifice to be tuned. Since each valve can be controlled separately, the orifice can be tuned off the center by changing the trajectory of the generated droplets downstream.
Abate et al. investigated the control of droplet formation further, using the same type of actuation [40]. The valves are incorporated in the orifice downstream of a flow-focusing junction. The droplet size decreases and the generation frequency increases with decreasing orifice cross section:

\[ d_d \sim \frac{\gamma d_o}{\eta_c \mu_c}, \]  

(2-11)

where \( d_d, \gamma, d_o, \eta_c \) and \( \mu_c \) are the droplet diameter, interfacial tension, orifice diameter, continuous phase viscosity and continuous phase velocity, respectively. However, the relationship (eqn (2-11)) remains empirical, as it is challenging to predict the cross section of the orifice deformed by the valves. Thus, the droplet size and the applied pressure have to be characterized experimentally.

Statically actuated valves tuning the channel geometry allow the control over a wider range of generation frequencies than the dynamically actuated counterparts. Dynamically actuated valves allow the generation on demand of virtually any polydisperse distribution droplet size with a characteristic time as short as the valve response time. Statically actuated valves cover a wider range of overall frequencies for a fixed monodisperse size. A similar concept was applied to a T-junction device using a horizontally moving valve [122]. The location of the valve is significant for the generation process. A valve located downstream of the junction easily reduces the droplet size. A valve placed upstream of the junction does not affect the droplet size.

Lee's group also used a statically actuated valve in the dispersed phase channel [115] (Figure 2-7(j)). The channel size is tuned by varying the negative actuation pressure. As the pressure in the actuation channel decreases, the channel cross section
increases, reducing the velocity of the dispersed phase. However, the streamlines become more concentrated at the centerline when the fluid exits the restriction with a higher velocity. In the flow-focusing configuration, the dispersed phase stream appears to be finer and consequently produces smaller droplets. Compared to other tunable designs, this device generates small droplets without deforming the channel excessively. Table 2.4 summarizes hydraulic/pneumatic control of droplet generation.

Table 2.4 Hydraulic/pneumatic control

<table>
<thead>
<tr>
<th>Source</th>
<th>Mechanism</th>
<th>Flow channel geometry</th>
<th>Fluids</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>[107]</td>
<td>Perturbation</td>
<td>T-junction: H = 20 or 80, W = 200</td>
<td>C = tetradecane + 1–3% Span 80 D = DI water + fluorescein</td>
<td>Perturbation frequency = 2–13 Hz *Side W is not stated</td>
</tr>
<tr>
<td>[108], [109]</td>
<td>Chopping</td>
<td>Flow focusing: H = 100, W = 60</td>
<td>C = triolein + polyglycerol-2 secaosystearate + PEG-10 polyglycerol-2 laurate D = DI water + 0.1% Vitamin C</td>
<td>Chopping frequency: &lt;17 Hz</td>
</tr>
<tr>
<td>[110]</td>
<td>Flow manipulation</td>
<td>T-junction: H = 25, W = 150</td>
<td>C = oleic acid/hexadecane/silicone oil + 3% Span 80 D = DI water</td>
<td>Dispensing time &gt;50 ms</td>
</tr>
<tr>
<td>[116]</td>
<td>Flow manipulation</td>
<td>T-junction: H = 11.5, 13.3, 15.3, W = 90</td>
<td>C = mineral oil D = water + 10% pigment solution/sodium alginate solution (0.1% or 0.05%)</td>
<td>Dispensing time &gt;40 ms</td>
</tr>
<tr>
<td>[111]</td>
<td>Flow manipulation</td>
<td>T-junction: H = 200, W = 200</td>
<td>C = oleic acid + 2.5% Span 80 D = water + diluted colored ink</td>
<td>Dispensing time &gt;50 ms</td>
</tr>
<tr>
<td>[112]</td>
<td>Flow manipulation</td>
<td>T-junction: H = 200, W = 200</td>
<td>C = mineral oil + 4.5% Span 80 + 0.4% Tween 80 + 0.05% Triton X-100 D = water + red dye</td>
<td>Dispensing time &gt;40 ms *Side W is not stated</td>
</tr>
<tr>
<td>[113]</td>
<td>Flow manipulation</td>
<td>T-junction: H = 200, W = 200</td>
<td>C = hexadecane + 2% Span 80 D = water</td>
<td>Dispensing time &gt;25 ms *Side W is not stated</td>
</tr>
<tr>
<td>[114], [121]</td>
<td>Geometry tuning</td>
<td>Flow focusing: H = 100, W = 100</td>
<td>C = DI water + 5% Triton X-100 D = olive oil</td>
<td>Droplet formation frequency &lt;14 Hz</td>
</tr>
<tr>
<td>[41]</td>
<td>Geometry tuning</td>
<td>Flow focusing: H = 50, W = 70</td>
<td>C = HFE-7500 fluoro carbon + 5% 1H,1H,1H,2H,2H-perfluoro-1-octanol + 1.8% fluoro surfactant ammonium carbonate D = water</td>
<td>Droplet formation frequency &lt;3000 Hz</td>
</tr>
<tr>
<td>[122]</td>
<td>Geometry tuning</td>
<td>T-junction: H = 100, W = 20</td>
<td>C = squalene oil + DOAB + negative-charged DNA modules D = DI water + 2.5% Tween 20</td>
<td>Droplet formation frequency &lt;4000 Hz</td>
</tr>
<tr>
<td>[115]</td>
<td>Geometry tuning</td>
<td>Flow focusing: H = 50, W = 85</td>
<td>C = olive oil D = DI water + 3% Triton X-100</td>
<td>Droplet formation frequency &lt;96 Hz</td>
</tr>
</tbody>
</table>

* H – height in μm; W – width in μm. b C – continuous phase; D – dispersed phase.
B. Piezoelectricity control

Piezoelectricity is used for a variety of applications such as sound/ultrasound generation, mechanical actuation, sensing and signal processing [123]. Piezoelectric actuation has also been used to control microfluidic droplet generation. The control approaches can be categorized according to the function of the piezoelectric element (Figure 2-8). For dispensing purposes, a piezoelectric actuator can be used to supply a fixed amount of dispersed phase for on-demand droplet generation as in ink-jet printing applications. On the other hand, piezoelectric actuation can disturb the interface between continuous and dispersed phases and affect the droplet generation process.

![Piezoelectricity control](image)

**Figure 2-8 Classification of the approaches for piezoelectricity control.**
(a) T-junction integrated with a piezo bimorph actuator [124].
(b) Flow focusing device with a piezoelectric disc to vibrate the oil–water interface formed during the droplet generation process [125], [126].
(c) Flow focusing device integrated with an interdigital transducer (IDT) to generate SAW [127].
(d) T-junction integrated with an IDT without the SAW influencing the oil–water interface. The dotted lines illustrate the changes after activation of the piezoelectricity control [128].
Dispensing droplets on demand

Xu et al. used piezoelectric actuation to dispense individual drops [124]. The T-junction device has a chamber filled with the dispersed phase (Figure 2-8(a)). The top of the chamber consists of a 180 μm-thick PDMS membrane, a 90 μm double-sided adhesive and a piezo bimorph actuator. An actuation pulse induces the energy that overcomes the interfacial tension and injects a droplet into the continuous phase:

\[ U_s = \pi d^2 \gamma, \]  

where \( d \) and \( \gamma \) are the diameter of the new droplet and the interfacial tension, respectively. The efficiency of the energy conversion from actuator to surface generation is about 0.9%, given that most of the energy is lost through viscous dissipation [124]. The induced energy is estimated as:

\[ W = \frac{3^2}{2 \times 8^2} d_{31}^2 E^2 Y V, \]  

where \( d_{31}, E, Y \) and \( V \) are the strain coefficient, applied electric field, elastic modulus, and volume of the piezo, respectively.

The droplet size is controlled by tuning the duration of the driving pulse and its voltage. Compared to the previously mentioned on-demand droplet generation approaches using pneumatic/hydraulic actuation, piezoelectric actuation is faster. While pneumatic/hydraulic actuation allows for 40 ms dispensing time, piezoelectric actuation can achieve 200 μs. The shorter dispensing time increases the generation frequency by more than two orders of magnitude. In addition to the simple one-by-one droplet dispensing mode, controlling the pulse patterns can generate droplets with different volumes. Dispensing a doublet or the generation of
two droplets per pulse was demonstrated. This droplet dispensing approach involves large, rapid movement of the oil–water interface, which may lead to the formation of satellite droplets. Furthermore, the duration of the device is challenged as large chamber deformations are required to produce a droplet. Bransky et al. used piezoelectric dispensing of individual droplets in a flow focusing configuration [129]. Compared to the T-junction configuration, the actuation chamber is smaller, as the flow-focusing configuration locally amplifies the pressure on the liquid interface. The smaller chamber also eliminates the generation of satellite droplets due to the smaller deformation at the oil–water interface. The location of the oil–water interface was adjusted by the height of each liquid.

Apart from attaching the piezoelectric actuator on the device for dispensing purposes, Shemesh et al. and Jakiela et al. also attached the actuator externally [130], [131]. The working principle is similar to the mechanism of flow manipulation using the electromagnetic valves (EMVs) discussed in the mechanical control section. Though, EMVs have much slower response compared to piezoelectric actuators [131].

**Mechanical vibration**

Piezoelectric actuation can be activated periodically to induce mechanical vibration on the oil–water interface. The droplet formation process is affected even if the vibration frequency is only about one order of magnitude higher than the droplet generation frequency. Cheung et al. used this approach to control droplet generation [125], [126]. The vibration is created by a piezoelectric disc attached on top of the dispersed phase channel (Figure 2-8(b)). Driven by a sinusoidal voltage with an amplitude of 100 to 250 V_{pp}, the piezoelectric disc vibrates with a frequency much
higher than that of droplet generation. The flow rates for both dispersed and continuous phases were fixed. For the whole set of flow rates tested in that study, the droplet size decreases with the vibration. The droplet size decreases with increasing applied voltage. The droplet size also decreases with increasing vibration frequency from 250 to 750 Hz. The faster advancement of the oil–water interface to the orifice leads to an earlier breakup of the interface and a smaller droplet size.

Ziemecka et al. used a similar setup to perturb an originally stable capillary jet formed at the flow focusing junction [132]. As the jet travels downstream, it breaks into droplets with sizes dictated by the imposed perturbation wavelength. Sometimes, the newly formed droplet may further break up into two daughter droplets as it moves downstream. Under a constant flow rate, the droplet size decreases with increasing vibration frequency $f_v$:

$$q_d = f_v N V,$$  \hspace{1cm} (2-14)

where $q_d$, $N$ and $V$ are the dispersed phase flow rate, the number of droplets in one cycle and the droplet volume, respectively. This model yields

$$d = \sqrt[3]{\frac{6q_d}{N\pi f_v}}.$$  \hspace{1cm} (2-15)

**Surface acoustic wave (SAW)**

In addition to the bulk transmission of vibration used in the approaches described above, surface acoustic wave (SAW) efficiently transfers acoustic energy along the surface of an actuator at its resonance frequency. SAW is excited by an interdigitated transducer (IDT), which is fabricated by patterning a pair of interdigitated comb-like electrodes on a piezoelectric substrate [127] (Figure 2-8(c)). The SAW
technology has been used in microfluidics applications such as mixing, separation, and droplet sorting [133].

Schmid et al. used SAW to control the droplet size generated by a flow focusing device under a constant set of flow rates [134]. The PDMS device with the microchannels is bonded directly to a flat piezoelectric LiNbO$_3$ substrate with the IDT (Figure 2-8(c)). The microchannel was aligned so that the IDT is located on the left side of the junction. The generated SAW travels along the microchannel for the continuous phase. The effect of the SAW on the droplet generation process is obvious upon activating the IDT. The SAW causes an asymmetric excitation of the thinning neck during the break up process (Figure 2-8(c)). Consequently, the time to breakup is shortened and reduces the droplet size. This phenomenon intensifies with increasing electric power. A stronger SAW shifts the neck and the droplet formation site further downstream with an even shorter break up time. A subsequent study on the control of droplet generation utilized SAW in a T-junction configuration [128]. The IDT is placed far away from the droplet formation site to prevent the SAW from affect the oil–water interface (Figure 2-8(d)). In this study, the fluids are driven by pressure.

The droplet length decreases with increasing SAW power. The authors hypothesized that the SAW promoted a pressure gradient in the continuous phase. This hypothesis was tested by comparing the pressure at the droplet formation site of the two cases: with and without SAW. The increase in pressure can be related to the volume force generated when SAW couples into the continuous phase [128]. The results of SAW-controlled droplet generation are close to that caused by the pressure increase in the continuous phase.
SAW control is an attractive approach as it allows contactless manipulation. The IDT can be located outside of the channel, preventing the electrodes from being in contact with the fluids directly. Furthermore, the IDT allows a small footprint suitable for integration into a small microfluidic device. However, the fabrication of the IDT requires a piezoelectric substrate, whose wetting properties might not be compatible with those of the channel material. Furthermore, a high-performance piezoelectric substrate could be expensive.

Collins et al. integrated a set of focused interdigital transducers (FIDT) on a modified T-junction device to enable on-demand droplet generation using SAW [135]. The curved gold electrodes were patterned with the focal point at the junction. The continuous phase was fed at a constant flow rate by a syringe pump, while the pressure of the dispersed phase was adjusted to form a static oil–water interface at the junction. A SAW pulse forced the dispersed phase into the main channel. On-demand droplet generation was thus controlled by manipulating the power and the duration of the SAW pulse. Table 2.5 summarizes piezoelectric control of droplet generation.
Table 2.5 Piezoelectric control

<table>
<thead>
<tr>
<th>Source</th>
<th>Mechanism</th>
<th>Flow channel geometry(^b)</th>
<th>Fluids(^a)</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>[124]</td>
<td>Dispensing</td>
<td>T-junction:</td>
<td>C = hexadecane</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(H = 50)–100, Main (W = 250)</td>
<td>D = water</td>
<td>Dispensing frequency &lt;2.5 kHz</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nozzle (W = 25)–100</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>T-junction:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(H = 50)</td>
<td>D = DI water</td>
<td>Dispensing frequency &lt;500 Hz</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Side (W) to channel</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>(W) ratio = (\frac{1}{4}) and 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flow focusing:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(H = 50)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Orifice (W) to channel</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(W) ratio = (\frac{1}{4}) and 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[125]</td>
<td>Mechanical vibration</td>
<td>Flow focusing:</td>
<td>C = mineral oil M5904 [light, 32 mPa s], paraffin oil 76335 (116 mPa s), mineral oil 310760 [heavy, 170 mPa s]</td>
<td>D = DI water</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(H = 100, ) main (W = 150)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Orifice (W = 40)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[126]</td>
<td>Mechanical vibration</td>
<td>Flow focusing:</td>
<td>C = mineral oil M5904 + 1.2% Span 80</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(H = 90, ) main (W = 130)</td>
<td>D = DI water</td>
<td>Vibration frequency = 200-500 Hz</td>
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<td></td>
<td></td>
<td>Orifice (W = 40)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[132]</td>
<td>Mechanical vibration</td>
<td>Flow focusing:</td>
<td>C = 20% polyethylene glycol (PEG) in water</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(H = 85, ) (W = 100)</td>
<td>D = 10% dextran in water</td>
<td>Vibration frequency = 2-50 Hz</td>
</tr>
<tr>
<td>[134]</td>
<td>Surface acoustic wave</td>
<td>Flow focusing:</td>
<td>C = HFE-7500 fluorocarbon + 1.8% DuPont Krytox 157</td>
<td>Actuation frequency = 161-171 MHz</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(H = 30, ) (W = 30)</td>
<td>D = water + bromophenol blue</td>
<td></td>
</tr>
<tr>
<td>[128]</td>
<td>Surface acoustic wave</td>
<td>T-junction:</td>
<td>C = HFE-7500 fluorocarbon + 1.8% DuPont Krytox 157</td>
<td>Actuation frequency = 160 MHz</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(H = 25, ) main &amp; side (W = 25)</td>
<td>D = water + bromophenol blue</td>
<td></td>
</tr>
<tr>
<td>[135]</td>
<td>Surface acoustic wave</td>
<td>T-junction:</td>
<td>C = olive oil [85 cP]</td>
<td>Actuation frequency = 48.4 &amp; 93.4 MHz</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(H = 30, ) main (W = 30)</td>
<td>D = water</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Side (W) = 20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) \(H\) = height in \(\mu\)m; \(W\) = width in \(\mu\)m; \(^b\) C = continuous phase; D = dispersed phase.
2.2.5. Other control methods

![Diagram of control methods]

**Figure 2-9 Other control methods**
(a and b) Flow focusing junction design with electrodes to control the flow rate of (a) the continuous phase [136] and (b) the dispersed phase [127].
(c and d) Flow focusing junction with trans-azoTAB photosensitive surfactant in the dispersed phase [137], (c) before and (d) after activation with UV light, and
(e and f) with cis-azoTAB in the dispersed phase (e) before activation (f) after activation with blue light.

*The dotted lines illustrate the changes after activation of the control.

A. Electrical control with electrorheological fluid
Electrorheological fluids (ERFs) are made of dielectric particles suspended in a carrier fluid [138]. Rheological properties such as viscosity and shear rate change if the fluid is subjected to an electric field [139]. Under an electric field, the suspended particles are polarized and aggregate along the field direction [138]. This feature allows the viscosity of the fluid to be electrically controlled, reversibly and continuously, even from the liquid to the solid state. ERFs have already found applications in automotive devices such as engine dampers and vehicle shock absorbers [139]. ERFs have been used in micropumps, microvalves and micromixers [136]. Giant electrorheological fluids (GERFs), a recently developed
kind of ERFs with much higher yield strength than the conventional ERFs, were also used in microfluidic devices [140].

Zhang et al. reported the use of GERFs for droplet generation in a microfluidic device [136]. Four fluid contacting electrodes were patterned near the droplet formation junction (Figure 2-9(a)). The electrodes were made of PDMS-based conducting composites loaded with black carbon nanoparticles. In that setup, the GERF was used as the continuous phase while water served as the dispersed phase. Before applying a voltage to the electrodes, long water plugs were formed stably at the junction. This process was interrupted by the application of a voltage on the electrodes, stopping the GERF flow. Only water flowed through the junction. If the voltage decreases to a critical value, the GERF rushes again into the junction, breaks the water stream and forms a new droplet. This process allows the generation of droplets to be controlled by the frequency. The same approach was used for a T-junction configuration with similar results. Niu et al. used GERF as the dispersed phase [141]. The carrier fluid of the GERF is sunflower seed oil, which is immiscible with the silicone oil used as the continuous phase. The fabrication of this device is similar to their previous one but the design was changed to suit this approach (Figure 2-9(b)), only 2 electrodes around the junction were used to control the dispersed phase.

Both control approaches using GERF are attractive as they provide a quick response time of less than 10 ms. The droplet size was determined by the applied frequencies and the shape of the control signal. This feature and control mechanism was adopted to perform 16 logic operations using KCl droplets as the signals [142]. However, given that the fluids are flow-rate driven by syringe pumps, the flow rates of GERF
and the other liquid should be carefully selected to prevent bursting of the channels or connections as the pressures build up in each fluid circuit. Another drawback is the concentration of the suspended dielectric particles in the GERF, which is over 20 wt% for effective control and might be too high for many applications.

B. **Photosensitive surfactant**

Photosensitive surfactants have been used to make droplet generation controllable by light. Diguet et al. introduced this control method using azobenzene trimethylammonium bromide surfactant (azoTAB) [143]. AzoTAB surfactant is available either in the trans or cis form under normal room light conditions. Trans(cis) form azoTAB converts to the cis(trans) form upon illumination of ultraviolet (UV) light [137]. The conversion is reversible, as they restore to their original forms once UV light is removed. The concept was demonstrated in a flow focusing configuration, where the dispersed phase is water with trans-azoTAB surfactant and the continuous phase is oleic acid. Initially, the flow rates are set to maintain a thin aqueous stream co-flowing with the continuous stream in the center (Figure 2-9(c)). After exposing the junction to UV light for about 2 s, the middle stream breaks and starts to generate monodisperse droplets (Figure 2-9(d)). As cis-azoTAB decreases the surface tension of the dispersed stream, the stability of the capillary jet changes to the dripping regime [144]. However, the transition does not happen at a high total flow rate, it is when the system is dominated by viscous forces [145].

Apart from the jetting/dripping transition, UV light was also used to modify the droplet size under normal room light conditions. As UV light is applied and surface tension increases, the droplet breaking site moves upstream, forming larger
droplets. With cis-azoTAB (see Figure 2-9), the dripping regime (e) turns into the jetting regime (f) after being exposed to blue light. This is consistent with the fact that the water stream with trans-azoTAB, which is converted by the blue light from cis-azoTAB, has a lower surface tension than the one with cis-azoTAB. Control with a photosensitive surfactant is easy to implement. The system does not require a high concentration of azoTAB to have a significant effect on control. However, the response to the control is slow as it takes seconds to reach a new stable state after the change in wettability by the respective light. Table 2.6 shows other active control methods on droplet generation.

Table 2.6 Other control methods

<table>
<thead>
<tr>
<th>Source</th>
<th>Control method</th>
<th>Flow channel geometry</th>
<th>Fluids</th>
<th>Notes</th>
</tr>
</thead>
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<td>[136]</td>
<td>Electrical control with electrohydrodynamic fluid</td>
<td>Flow focusing: ( H = 100, W = 200 )</td>
<td>C = sunflower oil based GER fluid, D = water</td>
<td>Voltage &lt; 1.5 kV, Frequency &lt; 250 Hz</td>
</tr>
<tr>
<td>[141]</td>
<td>Electrical control with electrohydrodynamic fluid</td>
<td>Flow focusing: ( H = 100, W = 200 )</td>
<td>C = silicone oil (70 cSt), D = sunflower oil based GER fluid</td>
<td>Voltage &lt; 1.5 kV, Frequency &lt; 250 Hz</td>
</tr>
<tr>
<td>[142]</td>
<td>Electrical control with electrohydrodynamic fluid</td>
<td>Flow focusing: ( H = 90, W = 200 )</td>
<td>C = silicone oil (100 cSt), D = sunflower oil based GER fluid</td>
<td>Voltage &lt; 450 V</td>
</tr>
<tr>
<td>[143]</td>
<td>Photosensitive surfactant</td>
<td>Flow focusing with orifice: Dispersed phase ( W = 90 ), Continuous phase and downstream ( W = 100 ), Orifice ( W = 33 )</td>
<td>D = trans-AzoTAB (12.6 mM) or cis-AzoTAB (44.6 mM) in water</td>
<td>UV illumination at ( i = 365 ) nm* Channel H is not stated</td>
</tr>
</tbody>
</table>

*\( H \) – height in \( \mu \)m; \( W \) – width in \( \mu \)m; \( C \) – continuous phase; \( D \) – dispersed phase.

2.3. Active control of bubble generation

Droplet generation with microfluidic device enjoys a wide range of methods to control the formation process of micro droplets and their size, as mentioned in section 2.2. The main reasons for this multiplicity of possibilities in droplet generation come from two fundamental facts: (i) the densities of both disperse and carrier phases are similar, and (ii) surface tension between two liquids is often very
small. Thus, any physical action fundamentally associated with the presence of the interface will efficiently affect both phases.

However, bubble generation involves two phases, one of which has a negligible density compared to the other, and surface tension between both is generally much larger than in liquid-liquid pairs. This, conversely, has two dramatic consequences: (i) externally induced interfacial effects are generally masked by a large surface tension, and (ii) interfacial actions fundamentally affect the liquid phase only (except in limited regions of the fluid domains). Therefore, the control of the bubble size using these devices is limited by a few parameters such as the flow rate, applied pressure and the geometry of the channel [109].

From the applications standpoint, there is a need to address this capability gap between bubble generation and droplet generation, which opens a very valuable niche for ingenuity. On the quest for a better control, some ideas to profitably focus some excess energy on the interface in similar configurations to enhance surface generation (i.e. a dramatic decrease in bubble diameter under the same flow rates and applied pressures) have been recently proposed, including swirl [146] or the use of patterned surfaces [70]. However, these proposals imply design and fabrication challenges that the microfluidic devices lack.

Therefore, a new method is needed to be developed to control bubble generation actively. Preferably, the method should be low-cost and does not involve complicated fabrication. The developed method should be able to enhance further the capability of microfluidic bubble generator to produce a wide range of bubble size.
2.4. Bubble acoustic

Bubbles expand and contract by acoustic pressure rarefaction and compression [147]. Driven by an acoustic pressure from an ultrasonic transducer system, bubbles rectify the rapid oscillation motion of the gas–liquid interface into steady streaming flow in the liquid around the bubbles [148]. This Rayleigh type of acoustic streaming is different from quartz wind streaming that operates in the MHz range [149]. The streaming flow has been used in microfluidic devices for mixing [150], pumping [151], sorting of microparticles [152] and sonoporation of suspended cells [153]. However, the application has not been used till to date to demonstrate in both bubble and droplet generation.

The bubbles that induce Rayleigh streaming in microfluidic devices are typically formed by passing liquids along blind side channels filled with gas [150]. These trapped semicylindrical air bubbles are difficult to form with a desired size as suitable wetting properties, blind side channel geometry and initial flow conditions are required. Furthermore, the bubble size will change after a period of continuous oscillation. Once the device has failed to create or maintain a bubble with a desired size, it needs to be replaced.

Instead of using an ultrasonic transducer system to oscillate the trapped bubbles with challenging repeatability, the system could be used instead to oscillate the gas-liquid interface formed during bubble generation. The oscillation by the system would affect the process of bubble formation and thus the generated bubbles sizes. As this phenomenon has not been studied before in the literature, it is worthwhile to investigate it systematically and find out if it can be developed as a new active control method for bubble generation.
2.5. Droplet/bubble measurement

Existing fluid flow measurement technology, micro-particle image velocimetry (μ-PIV) can be adopted as the measurement system for droplet/bubble generation. However, it requires fluorescing particles [42], which may not be suitable for some biological applications. Measurement using laser is label-free and easy to set up, but the obtainable parameters is limited, which is generally used to measure the production frequency of droplet/bubble [43].

Measurement can also be done on a traveling droplets/bubbles video. Under normal circumstances, the outline of droplet/bubble is usually continuous and darker than the background [44]. This allows the recognition of droplet/bubble contours frame by frame through video processing software. This measurement system is especially useful for droplet/bubble generation as it is label-free while able to obtain many different parameters from the collected contour data during video processing. The software for the measurement system was recently released by Basu, which is called Droplet Morphometry and Velocimetry (DMV) [44]. The software is able to obtain parameters such as area, centroid position, velocity and orientation of motion.

However, the only publicly available software application, DMV, is not fully automated. Also, the processing speed of DMV is not quick enough to reflect the result of the measurement on droplet/bubble instantly or in situ. In situ measurement system can serve as an invaluable tool to study droplet/bubble generation efficiently. It also allows users to implement immediate changes to rectify any abnormalities detected. Therefore, development of a new video processing software application that is fully-automated and high throughput is needed to achieve in situ measurement on droplet/bubble.
2.5.1. **Background extraction operation (BEO)**

For video processing, background extraction operation (BEO) is the first basic step in the image processing for subsequent droplet measurements. Indeed, tracking the moving object (droplet/bubble) accurately by the video processing software requires a background removal operation (BRO), which uses the extracted background by BEO [154]. By using a properly extracted background, BRO clears out permanent background features such as the channel wall from every frame in the video. This eliminates the need for manual intervention to prevent those features from being tracked by the software.

BEO can be done by simply taking the picture of the channel before the formation of droplet. However, the extracted background becomes obsolete once the image condition changed. For instance, a new BEO has to be performed once the stage position or the lightning condition changes. New BEO is also needed when the device has been used for an extended period of time, as the channel walls may swell and deform due to the presence of organic solvents [155].

It is inconvenient and inefficient to stop the droplet formation process just for a new BEO whenever there is a change in the image condition. Fortunately, each of the video frames captured already contains fractional information regarding the background image. Therefore, it is possible to perform BEO from a video, by combining the fractional information from several number of video frames in order to form a complete background image.

Basu suggested a statistical survey approach on each of the pixels in the video frames in order to perform BEO from a video [44]. By performing the survey, the
statistics of the intensity value for each of the pixels across the sampled frames can be determined. A new image can then be formed by setting each of the pixels with the intensity value accordingly to the statistical data such as average, mode, median, minimum and maximum.

According to Basu, the new image generated by setting the intensity values to their modes can represent the background correctly for most of the time [44]. However, it was found that this method is not ideal for all situations as it fails to extract a proper background for droplet generated at low flow rate ratio (dispersed phase to continuous phase), where the droplets occupied the channel for more than half of the time. Hence, a new software application to address this limitation is needed.

2.5.2. Binary threshold value selection (BTVS)
For video recognition, an image is usually converted into a binary image for contour recognition. Before binary image conversion, there is a need to select a suitable binary threshold value as it affects the quality of the contour recognition. The suitable binary threshold value is unique for each video as the channels are captured under a variety of lightning condition, background and degree of transparency. This makes the video, from one to the other, to have different edge contrast of droplets outlines [156]. Therefore, binary threshold value selection (BTVS) is another essential operation in the processing step. The operation ensures the exact contours to be recognized from the converted binary image, and that conforms closely to the outlines of droplets. BTVS is also vital for the reliability of droplet tracking and the accuracy of the droplet measurement. By using the optimal threshold value, the need to perform dilation or closing operation [157] could also be reduced to join the disconnected traced droplet outlines from an improperly processed binary image.
The currently available droplet measurement software or DMV requires the user to perform BTVS manually. This is done by changing the value of binary threshold and checking the converted binary image [44].

2.5.3. Video recognition performance
DMV is written in MATLAB [44]. The software has not been optimized for high performance as mentioned by Basu. Apart from MATLAB, Open Source Computer Vision (OpenCV) is another popular image processing library. OpenCV library is written in optimized C and its performance can be enhanced by the use of multicore processors [158]. Due to this, OpenCV consumes lesser CPU time than MATLAB for basic image processing operations [159]. OpenCV is also widely used and adopted in many research fields as the tool for real-time computer vision that requires high processing speed [160]. Therefore, by adopting OpenCV library for video processing in droplet/bubble measurement, the performance of video processing could be improved significantly.

2.6. Proposed research scope
Based on the review, there are already numerous methods to control the process of droplet generation actively. The methods could be categorized according to the type of energy such as electric, magnetic, mechanical and temperature. Bubble generation, on the other hand, has much fewer active control methods. Also, the available methods are challenging to design and fabricate in microfluidics. As gas-liquid interface are formed during bubble generation in microfluidic device, bubble acoustic could be the potential method to be discovered and developed to control the generation actively. The gas-liquid interface vibration induced by ultrasonic
transducer could affect the behavior of bubble generation, thus allows the control to
the size of the generated bubble.

For measurement of droplet/bubble, video processing is the most suitable method
to evaluate the capability of newly developed active method quickly and efficiently.
Video processing does not need fluorescent particles. Besides, many parameters
such as area, centroid position, velocity and orientation of motion could be obtained
through video analysis. DMV by Basu is not fully automated as user needs to perform
background extraction operation (BEO) manually by selecting a correct statistical
scheme. Additionally, user needs to select a suitable binary threshold value to
ensure reliable tracking and accurate measurements of droplets. The requirements
of the two user interventions for the software, plus slow in the video recognition
performance, impedes its capability to perform in situ measurement.

Based on the review, the research scope for this thesis is listed as follow:

1. Design and development of video processing software based on OpenCV to
measure droplet/bubble generation. This is to improve the video processing
performance of the currently available software.

2. Implementation of full automation in video processing software by
developing algorithms for automated background extraction and threshold
value selection.

3. Development of ultrasonic transducer system that is cost-effective while
powerful enough to vibrate gas-liquid interface.

4. Design and fabrication of microfluidic device for bubble generation that is
compatible for incorporation of ultrasonic transducer system. The integrated
device should allow study of the control effect by an ultrasonic transducer to
the bubble generation under a microscope fitted with high speed camera.
The following research roadmap illustrates the flow of the proposed research.

<table>
<thead>
<tr>
<th>Fabrication of microfluidic device</th>
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<tr>
<td>• Evaluation of channel fabrication and bonding methods</td>
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<td>• Selection of PMMA or PDMS device</td>
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<tr>
<th>Generation of droplet and bubble</th>
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<tr>
<td>• Interconnection setup</td>
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<tr>
<td>• Characterization of bubble/droplet generation</td>
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<tr>
<th>Development of video processing software</th>
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<tbody>
<tr>
<td>• Based on OpenCV library</td>
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<tr>
<td>• Developing algorithms for automation:</td>
</tr>
<tr>
<td>o background extraction operation</td>
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<tr>
<td>o binary threshold value selection</td>
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<tr>
<td>• Integrating with camera SDK for <em>in situ</em> measurement</td>
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<table>
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<tr>
<th>Development of new active control method</th>
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<tbody>
<tr>
<td>• Setup of ultrasonic transducer system</td>
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<tr>
<td>• Integration of microfluidic device to the system</td>
</tr>
<tr>
<td>• Study on the characteristic of the new control method</td>
</tr>
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</table>
Chapter 3: Fabrication and testing of a functional PMMA droplet generator

In this chapter, fabrication of a poly(methyl methacrylate) (PMMA) droplet generator by injection molding is discussed. The fabricated PMMA device was tested under different sets of flow conditions (i.e. flow rates) to examine its capability as a droplet generator. The droplet formation patterns were also further characterized to ascertain the performance of the generator.

3.1. Fabrication of PMMA device by injection molding
As mentioned in appendix, laser has been used to engrave the channel of microfluidic device. However, it was found that the fabrication quality was unsatisfactory for droplet generation. Therefore, injection molding is used as another method to fabricate the PMMA device. The process flow of the fabrication is illustrated in Figure 3-1.

The fabrication method involved making of primary and secondary molds. The primary mold was made by micromachining on a silicon wafer using deep reactive-ion etching (DRIE). The primary mold was then used to produce secondary mold by hot embossing on a bulk metallic glass (BMG) plate. The BMG mold was then used as a mold insert for injection molding.
The micrographs in Figure 3-2 were taken from (a) the primary silicon wafer mold (b) the secondary BMG mold, and (c) the injection molded PMMA with BMG mold as the insert. The fabrication process of each part will be explained further in the subsequent subsections.
3.1.1. Primary mold fabrication – Micromachining on a silicon wafer

Micromachining process started by performing hexamethyldisilazane (HDMS) priming on a clean silicon wafer (4 inch, <100> type). Priming was done in order to promote adhesion between photoresist and the polished surface of the wafer. Afterwards, a thin layer (~7 µm) of positive photoresist (AZ 9260, Microchemicals GmbH) was deposited on the primed wafer by spin-coating (500 rpm, 8 s followed by 4000 rpm, 30 s [Figure 3-1(a2)]. After soft-baking (100°C, 4 minutes) and cooling down the coated wafer to room temperature, microfluidic channels were patterned using ultraviolet light (365 nm) through a photomask [Figure 3-1(a3)]. The duration of the exposure was 40 s under light intensity of 9.3 mW/cm². The photomask was fabricated on plastic with 16,000 dpi. The design for the photomask is shown at the center of Figure 3-3.

After developing the photoresist [Figure 3-1(a4)], the areas which are not covered by the photoresist were etched by deep reactive-ion etching (DRIE). The power of the radio frequency (RF) was set to 800 watt for the DRIE process. For each cycle of etching and passivation, the etching duration by SF₆ and O₂ gasses is 8 s, while the passivation duration by C₄F₈ gas is 10 s. The number of cycles for etching and passivation was determined after measuring the rate of etching from the initial 10 cycles. After the process, the channels were engraved on the silicon wafer [Figure 3-1(a5)], which are shown in the scanning electron microscope (SEM) micrographs in Figure 3-3 (after removing the remaining photoresist).
3.1.2. Secondary mold fabrication – Hot embossing on BMG plate

After dicing the micromachined silicon wafer, it was used as the primary mold to replicate the structure through hot embossing process on a plate made of Zr-based bulk metallic glass (BMG). This method of fabricating secondary mold has been done by Tor’s group to produce the mold for PMMA injection molding of micromixer [161] and droplet generator [162]. The process was done on a pair of electronically controlled heated platens [Figure 3-1(b1)]. The compression force to the platens was controlled using an Instron machine. The platen was heated gradually until slightly higher than the glass transition temperature \( T_g = 350-360^\circ C \) of BMG. Afterwards, the mold and the BMG were compressed through the heated platens for about 2 minutes [Figure 3-1(b1)]. After releasing the pressure, the compressed mold and BMG plate were removed and cooled at room temperature naturally. The remaining parts of the mold adhered to the BMG plate were then removed by etching.
in stirring diluted potassium hydroxide (30% w/v KOH) at 80°C [Figure 3-1(b3)]. Figure 3-4(a) shows the hot embossed BMG plate after separating from the primary mold. The BMG plate was then wire-cut into a 25x25 mm square so to be inserted into the injection molding tool. The inlets and outlets of the channels were then drilled. Figure 3-4(b) shows the BMG plate after being wire-cut and drilled for holes.

![Figure 3-4 (a) BMG plate right after separating from primary mold (b) BMG plate after being wire-cut and drilled for holes.](image)

### 3.1.3. Plastic injection molding - the making of PMMA device

The secondary mold was mounted on a generic development platform, which had been established for PMMA injection molding. The mold of the platform has a replaceable mold insert where the microfluidic pattern is created. When the mold is fitted with a blank insert, it produces a blank substrates with interconnect ports only (Figure 3-5). The interconnect ports, as shown in Figure 3-5, are made to facilitate tubing installation.

![Figure 3-5 Blank generic development substrate.](image)
By replacing the blank insert with the secondary mold, a device with microchannels connected the interconnection ports were produced. PMMA LG2 (SUMIPEX, Sumitomo Chemical) was used as the injection molding resins. The parameters used for the injection molding are listed in Table 3.1, while Figure 3-6 shows the photo of an injection molded PMMA device.

Table 3.1 Parameters for injection molding

<p>| | | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>1</td>
<td>Pre-drying</td>
<td>4 hours under 70°C</td>
</tr>
<tr>
<td>2</td>
<td>Melt temperature</td>
<td>260°C</td>
</tr>
<tr>
<td>3</td>
<td>Mold temperature</td>
<td>75°C</td>
</tr>
<tr>
<td>4</td>
<td>Injection volume flow</td>
<td>32 ccm/s</td>
</tr>
<tr>
<td>5</td>
<td>Injection pressure</td>
<td>1000 bar</td>
</tr>
<tr>
<td>6</td>
<td>Holding pressure</td>
<td>580 bar</td>
</tr>
<tr>
<td>7</td>
<td>Holding time</td>
<td>8 s</td>
</tr>
<tr>
<td>8</td>
<td>Cooling time</td>
<td>25 s</td>
</tr>
</tbody>
</table>

Figure 3-6 Injection molded PMMA device.

3.2. Bonding of channel

The droplet generator fabricated by injection molding cannot be used to perform droplet generation directly because the top parts of the channels are open. The substrate needs to be bonded with another planar object to enclose the open channels. In the following subsections, both thermal and adhesive tape bonding are discussed.

3.2.1. Thermal bonding on PMMA device

Several attempts have been made to enclose the channels with another PMMA cover plate using thermal bonding machine. However, the attempts were unsuccessful
mainly because the injection molded part had significant residual stress which was built up during the injection molding process. This caused annealing process to take place concurrently with the thermal bonding process on the molded part. The annealing process can distort the geometry and the surface evenness of the channel, as reorientation of the molecules took place [163]. This makes thermal bonding process challenging to achieve optimum bonding especially on the molded part with variety of residual stresses across the areas. Figure 3-7 shows the samples of the unsatisfactory bonding result that occurs on the same chip: (a) overbonding which blocks the flow of fluid, (b) underbonding which makes fluid to seep through the unbonded seams. The bonding was done under pressure of 0.8 MPa for 5 minutes at 85°C.

![Figure 3-7 (a) Overbonding and (b) underbonding of channels.](image)

### 3.2.2. Adhesive tape bonding on PMMA device

Adhesive tape bonding was used as a fast and easy way to enclose the channels on device. This method has been used to bond PDMS [164] and PMMA [165] microfluidic device. It is attractive as it is flexible to conform to the slight unevenness of the injection molded surface. Furthermore, this bonding does not involve heat that could deform the geometry of channel. However, the requirement on the tape adhesive is stringent for droplet generator as it has to be resistant to
both oil and water. To the best of our knowledge, there is no reported PMMA device bonding with tape adhesive that is resistant to both oil and water. Therefore, a study was done to find out a suitable adhesive tape to bond the PMMA device. One of the important tests involves pressure test on the channel bonded with different type of tapes.

Figure 3-8 shows the setup of the pressure test. The pressure test was started by injecting liquid from syringe 2 into the channels of the tape bonded PMMA device. Two different liquids, DI water or mineral oil (330779, Sigma Aldrich; viscosity $\eta=21.7$ mPa s), were used separately for the pressure test. This is because both liquids are used for the droplet generation. The bonding strengths can be different when the tape is in contact with liquids of different polarities. Once the liquid had filled up about 10% of the buffer chamber from its bottom, the syringe was locked to its position. Syringe 1 and a pressure gauge (Type 2086, Ashcroft) were connected to the top of the buffer. Syringe 1 was used to apply pressure to the liquid through the buffer chamber while the pressure gauge was used to measure the applied pressure.

![Figure 3-8 Schematic for the pressure test.](image)

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During the test, the plunger of syringe 1 (309653 - 60 mL, Becton Dickinson) was actuated by a syringe pump (Model 100, KD Scientific) at a constant linear speed of 0.21 mm/s. Whenever a decrease in the measured pressure value was noticed, the actuation was paused. The maximum pressure achieved before that was then noted. Figure 3-9 shows the result of the pressure test done on the bonded PMMA device by different adhesive tapes.

![Figure 3-9 Result of the pressure test.](image)

From the figure, all the tapes except 3M Polyester 853 have lower pressure when the tape is in contact with oil compared to water. This is particularly obvious for Panfix Self Adhesive Cellulose. For the tape, the maximum pressure with water is comparable to 3M Polyester 853 but its pressure in oil is much lower than a quarter of the pressure in water. Most of the tapes have poorer bonding under the presence of oil. This is because their adhesive is easily dissolved by oil, which weakens and decreases the maximum pressure withstand-able by the bonding.
Out of the tested tapes, 3M Polyester 853 appears to be the best among all, for both water and mineral oil. From the technical data, 3M Polyester 853 is made of acrylic adhesive. The tape is resistant to many solvents including jet engine fuel, hydraulic fluid, and lubrication oil. Together with other factors such as residue free after peeling for reuse of the device, optically clear enough for droplet measurement, the tape was used to bond the device.

3.3. Droplet generation in PMMA device
PDMS is the most popular substrate material used for droplet based generation. The capability of droplet generation in PDMS devices (plasma bonded to glass slide) has already been evaluated by many workers in this field.

However, due to the high cost involved in fabricating PMMA devices, droplet generation by PMMA devices is rarely reported in the literature. In the following sections, the PMMA device (bonded by adhesive tape) was tested to understand the capability and the limitation of such device.

3.3.1. Channel design and fluid flow setup

![Diagram of channel design fluid flow setup of PMMA device](image)

Figure 3-10 (a) channel design fluid flow setup of PMMA device (b) micrograph of the produced droplet.

The PMMA device has a cross-junction design, with channel width and height of 200 μm. The channel design is shown in Figure 3-10(b). Water droplets in oil was formed by flowing DI water to the central channel of the cross junction and mineral
oil (330779, Sigma Aldrich; viscosity $\eta = 21.7$ mPa s) with 1% w/w non-ionic surfactant (Span 80, Sigma Aldrich) to the two side channels. A syringe pump (Model 200, KD Scientific) was used to provide a constant volumetric flow rate for the water and oil. The equilibrium interfacial tension (water-oil) is 27.8 mN/m. The surfactant was added to reduce interfacial tension, to prevent coalescence of droplets, and to promote wetting of the continuous phase.

3.3.2. Interconnections

The interconnections from syringes to the access ports on PMMA device were aimed to be glue-free while resisting to adequate amount of pressure. This is to ensure a fast & easy test setup and to prevent possible contamination from glue. The target was achieved by tight fitting on access ports using right size Tygon tubing. However, as Tygon tubing is flexible, tubing length could not be too long as it could extend the time for flow to stabilize. Therefore, semi-rigid polytetrafluoroethylene (PTFE) tubing was used as the intermediate tubing connecting Tygon tubing to syringe. Different combinations of adapters were tested to ensure robust connections between PTFE and Tygon tubing, between PTFE tubing and syringe. After perfecting
the configuration of the setup, a new, reliable flow test preparation could be performed in less than an hour (Figure 3-11).

3.3.3. Syringe pump setup

Flow rate ratio is defined as the ratio of the dispersed phase flow rate $Q_d$ to the continuous phase flow rate $Q_c$. Flow rate ratios can be varied using two syringe pumps. This setup allows each of the pumps to control the flow rate from syringe independently. Flow rate ratios can also be varied by using a dual syringe pump. For the syringes fitted in the setup, the plungers of both syringes are pushed by a common actuator. By using syringes with different cross section areas, the flow rate is different from each syringe. Thereby, the flow rate ratio can be set by paring the syringes of different sizes.

Initial evaluation on the droplet generation results has found that, the coefficient of variation (CV) of the droplet size was generally higher for two pumps setup (>5%) compared to a dual syringe pump setup (around 3%). Therefore, the results reported here are all from dual syringe pump setup. The syringes used in the setup were 1, 3, 5, 10 mL of Becton Dickinson Plastipak with Luer-Lok. The syringe sizes and the respective diameters are listed in Table 3.2.

<table>
<thead>
<tr>
<th>Syringe size (mL)</th>
<th>Diameter, D (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.70</td>
</tr>
<tr>
<td>3</td>
<td>8.59</td>
</tr>
<tr>
<td>5</td>
<td>11.99</td>
</tr>
<tr>
<td>10</td>
<td>14.48</td>
</tr>
</tbody>
</table>

The flow ratios used in the setup with the paired syringe sizes are listed in Table 3.3.

The flow rate ratio is calculated by using
\[
\frac{Q_d}{Q_c} = \frac{D_w^2}{D_o^2},
\]

where \(D_w\) is the diameter of the water syringe and \(D_o\) is the diameter of the oil syringe.

<table>
<thead>
<tr>
<th>Flow rate ratio, (Q_d/Q_c)</th>
<th>Syringe size – Water (mL)</th>
<th>Syringe size – Oil (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.11</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>0.35</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>0.68</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>1.00</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>1.46</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>2.84</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>9.49</td>
<td>10</td>
<td>1</td>
</tr>
</tbody>
</table>

**Table 3.3 Flow ratios with the paired syringe sizes**

### 3.3.4. Surfactant

Droplet generation was first tested using pure mineral oil without surfactant. At low flow rate of \(Q_o = 10 \, \mu\text{L/min}\), flow rate ratios of 0.68 and 0.35, the length of the produced droplet is very long. Moreover, the droplet length and the generation frequency at the junction are highly irregular. The produced droplet tends to stick to the tape at the downstream channel, where they coalesced frequently with the newly generated droplet. Figure 3-12 shows an instant of the droplet generated at the junction at flow rate of \(Q_o = 10 \, \mu\text{L/min}\) with different flow rate ratio: (a) 0.68 (b) 0.35.

![Figure 3-12 Instance of the droplet generation at the junction at flow rate of \(Q_o=10 \, \mu\text{L/min}\) with flow rate ratio of (a) 0.68 (b) 0.35.](image)

Stratification still occur throughout the downstream channel even after the flow rate of syringe pump was increased progressively until \(Q_o = 90 \, \mu\text{L/min}\). At the highest
flow rate, water phase adheres to one side of the channel. This continues throughout the downstream channel until the outlet of the device. Figure 3-13 shows the stratification of water phase around the junction at flow rate of $Q_o = 90 \, \mu L/min$ with different flow rate ratio: (a) 0.68 (b) 0.35.

![Figure 3-13 Instance of the droplet generation at the junction at flow rate of $Q_o=90 \, \mu L/min$ with flow rate ratio of (a) 0.68 (b) 0.35.](image)

After adding 1\% w/w surfactant (Span 80, Aldrich) to the mineral oil, monodispersed droplets can be formed more easily at the junction. Figure 3-14 shows the droplets formed at different flow conditions: (a) $Q_o = 10 \, \mu L/min$, flow rate ratio=0.68 (b) $Q_o = 10 \, \mu L/min$, flow rate ratio=0.35 (c) $Q_o = 70 \, \mu L/min$, flow rate ratio=0.68 (d) $Q_o = 90 \, \mu L/min$, flow rate ratio=0.35.

![Figure 3-14 After adding surfactant monodispersed droplets are formed more easily under different conditions: (a) $Q_o=10 \, \mu L/min$, flow rate ratio=0.68, dripping regime (b) $Q_o=10 \, \mu L/min$, flow rate ratio=0.35, dripping regime (c) $Q_o=70 \, \mu L/min$, flow rate ratio=0.68, jetting regime (d) $Q_o=90 \, \mu L/min$, flow rate ratio=0.35, jetting regime.](image)

Monodispersed droplets can be form more easily after the addition of surfactant. This can be related to the reduction in the surface tension. The formed droplets are hindered from coalescence under presence of surfactant as the droplets are stabilized by steric repulsion of the surfactant molecules. Also, as the continuous phase flows along the droplet interface, surfactant gradients are induced. This create
a force acting against the drainage of two immediate droplets, also known as “Marangoni effect” [166]. It is also believed that the surfactant helps to reduce the adhesion between the dispersed phase and the adhesive tape. This allows a clean separation of the dispersed phase at the junction and also reduces the drag by the tape on the generated droplets [167].

3.3.5. Multiphasic behavior at the junction

![Image](https://example.com/image.png)

Figure 3-15 Multiphasic behavior (a) dripping (b) jetting (c) threading (d) varicose confinement (e) stratification (f) tubing.

Figure 3-15 shows different multiphasic behaviors at the junction under variety of flow rates. The behaviors are summarized in the graph using capillary numbers. The equations for the capillary numbers are as follow

\[ Ca_d = \frac{\eta_d l_d}{\gamma_{cd}}, \quad Ca_c = \frac{\eta_c l_c}{\gamma_{cd}}, \]  

(3-2)

where \( \eta_d \) and \( \eta_c \) are the viscosities for dispersed and continuous phases respectively, \( \gamma_{cd} \) is the interfacial tension between the dispersed phase and the
continuous phase. \( J_d = Q_d/w^2 \) and \( J_c = Q_c/w^2 \) are the superficial velocity for dispersed phase and continuous phase respectively.

**A. Dripping**

Monodisperse droplets are generated by dripping. The generation starts by formation of a liquid finger at the junction of the dispersed phase. The finger then expands towards the downstream channel. After it reaches the downstream channel, the side channels are obstructed by the dispersed phase finger. As the continuous phase is still being pushed to the side channels at a constant flow rate, pressure built up at the side channels. The built-up pressure starts to pinch the dispersed phase finger at the junction. This causes the necking of the finger which leads to the eventual breakup of the finger and become a new droplet. The breaking of the dispersed phase occurs around the junction. The tip of the dispersed phase usually contracts right after the breaking of the dispersed phase. The length of the droplet formed by dripping is the function of \( \alpha_cCa_c \), where \( \alpha_c = Q_c/(Q_c + Q_d) \) [168]. The droplet formed by dripping is shown in Figure 3-15(a), taken at \( Q_c=20 \mu\text{L min}^{-1}, Q_d=7\mu\text{L min}^{-1} \).

**B. Jetting**

Jetting occurs at higher flow rate than dripping. A thread is formed during jetting from the junction to part of the downstream channel. Due to the instability of the thread, varicose instability is formed at the end of the thread. The varicose grows and breaks into droplet eventually. The droplets formed under jetting are still regular in size. The tip of the thread does not contract significantly after the formation of droplet. The droplet length formed by dripping is the function of the flow rate ratio \( Q_d/Q_c \) [168]. As stated in the literature, jetting occurs when \( Ca_d \) is
higher than 0.1. However, it is observed from the result that jetting occurs at $Ca_d$ as low as $1.3 \times 10^{-4}$. This is due to the adherence of the dispersed phase to the adhesive tape. The droplet formed by jetting is shown in Figure 3-15(b), taken at $Q_c=150 \ \mu L \ \text{min}^{-1}$, $Q_d=16.5 \ \mu L \ \text{min}^{-1}$.

C. **Threading**

Threading happens when $Ca_d$ is much larger than 0.1 [168]. However, the adherence of the dispersed phase to the adhesive tape makes threading happens at $Ca_d$, as low as 0.0107. Threading happens around flow rate ratio of 1. It starts by the adherence of the dispersed phase to the tape. This forms a long thread along the downstream channel. No pressure was built up at the side channel as there are still spaces around the thread to allow co-flowing of continuous and dispersed phase. No droplet is formed from the thread unless perturbation is done on to the device. Figure 3-15(c) shows the threading at downstream channel, which was taken at $Q_c=80 \ \mu L \ \text{min}^{-1}$, $Q_d=80 \ \mu L \ \text{min}^{-1}$.

D. **Varicose confinement**

The thread in this stage is much thicker than threading. The geometrical confinement by the presence of walls contributes to the deformation of the thread [169]. Along the thread, there are multiple nodes. The adhesion of the thread to the tape prevents the thread from being broken by the confined flow of the continuous phase. This makes the varicose oscillates as if waves in motion. Figure 3-15(d) shows the varicose confinement behavior, which was taken at $Q_c=40 \ \mu L \ \text{min}^{-1}$, $Q_d=114 \ \mu L \ \text{min}^{-1}$.
E. Stratification

At a very high $Q_d$ compared to $Q_c$, stratification occurs. Under this situation, the wetting effect is higher than the lubricating effect at the channel. This makes the dispersed phase or the formed droplets sticks to the wall and leave small droplets of higher curvature on the walls [168]. The small droplets could be collected by the future tip of the phase or the future droplets. In this case, the dispersed phase adheres to one side of the channel, forming a biphasic co-flow. Droplets do not form from under the situation. Figure 3-15(e) shows the instance of stratification, which was taken at $Q_c=40$ μL min$^{-1}$, $Q_d=380$ μL min$^{-1}$.

F. Tubing

This behavior occurs at very low $Q_c$ and $Q_d$ [168]. When dispersed phase first passed through the junction, apart from entering into the downstream channel, it invades also to the side channels. This is because the pressure at the side channel is still relatively low at the moment. During the invasion, the pressure at the side channels build up slowly as the dispersed phase is blocking the flow of the continuous phase at the side channels (left inset of Figure 3-15(f)). Eventually, the built up pressure squeezes the dispersed phase out from the side channels (right inset of Figure 3-15(f)), breaks up the dispersed phase and allows the flow of continuous phase through the junction. After releasing the built-up pressure, the process restarts where the dispersed phase invades the lower pressure side channels again. The produced droplets are long in length, which can be extended up the outlet of the device. The insets in Figure 3-15(f) were taken at $Q_c=2$ μL min$^{-1}$, $Q_d=0.22$ μL min$^{-1}$. 
3.3.6. Droplet generation results

Quantitative results were taken only from droplets generated under dripping and jetting as they are more regular in size. For every change in the flow rate, 20 minutes was given for the process to stabilize. Afterwards, measurement of a droplet length is done repeatedly at 5 seconds interval. For every set of flow rates, 100 droplets were taken.

Generally, the length increases as the flow rate ratio $Q_d/Q_c$ increases. On the other hand, the increase in total flow rate decreases the length for most of the time. Within the same flow rate ratio, the length decreases linearly at low total flow rate. Once the total flow rate is larger than a certain value, the change in length becomes relatively low as the total flow rate increases.

Figure 3-16 shows the graph of the measurement result. Based on the result, the trend of the data points can be divided into two regions. In the first region, the data points decrease linearly within the same flow rate ratio set as $Q_d$ increases. In the second region, the change in data points moves little within the same flow rate ratio. The changes are relatively small compared to the changes in the first region with the change in $Q_d$. 
By observing the video of the droplet generation process at each set of flow rates, it was found that the droplets generated in the first region are by dripping. The droplet lengths in dripping region is found to be related to $\alpha_c Ca_c$, where the data collapse into a master curve, as shown in Figure 3-17. For the second region, the droplets are

Figure 3-16 Measured droplet length under different sets of flow rates and ratios.

Figure 3-17 Data from dripping region. L is length of droplet while w is width of channel.
generated by jetting. The droplet lengths in this region are related to $Q_d/Q_c$, as shown in Figure 3-18. In another words, the length does not change much as the total flow rate changes within the same flow rate ratio. The micrographs at the selected sets of flow rates are shown in Table 3.4.

![Figure 3-18 Data from jetting region. L is length of droplet while w is width of channel.](image)

**Table 3.4 Micrographs at selected sets of flow rates**

<table>
<thead>
<tr>
<th>Flow ratio</th>
<th>$Q_d$ (μL min⁻¹)</th>
<th>Dripping</th>
<th>$Q_d$ (μL min⁻¹)</th>
<th>Jetting</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.11</td>
<td>4.4</td>
<td><img src="image" alt="Micrograph" /></td>
<td>16.5</td>
<td><img src="image" alt="Micrograph" /></td>
</tr>
<tr>
<td>0.35</td>
<td>7.0</td>
<td><img src="image" alt="Micrograph" /></td>
<td>24.5</td>
<td><img src="image" alt="Micrograph" /></td>
</tr>
<tr>
<td>0.69</td>
<td>13.8</td>
<td><img src="image" alt="Micrograph" /></td>
<td>34.5</td>
<td><img src="image" alt="Micrograph" /></td>
</tr>
<tr>
<td>1.00</td>
<td>20.0</td>
<td><img src="image" alt="Micrograph" /></td>
<td>40.0</td>
<td><img src="image" alt="Micrograph" /></td>
</tr>
<tr>
<td>1.46</td>
<td>14.6</td>
<td><img src="image" alt="Micrograph" /></td>
<td>58.4</td>
<td><img src="image" alt="Micrograph" /></td>
</tr>
</tbody>
</table>
3.3.7. Discussion

The fabricated PMMA droplet generator is able to generate droplet with variety of lengths by adjusting the flow rates of liquids. Monodispersed droplets can be generated either by dripping and jetting under a limited range of flow rates. Due to the use of adhesive tape, the jetting regime forms at a much lower $Ca_d$ than the stated value (>0.1) in the literature. Under high total flow rate, the dispersed phase adheres to one side of the channel due to adherence to the adhesive tape. Thus, the PMMA device is unable to produce a droplet size that is much smaller than the channel width, which could have been produced under the jetting regime of higher total flow rate. Therefore, an alternative fabrication method and material is needed.

Apart from this limitation, the fabricated device still has its own merits. PMMA is more compatible to wider range of organic solvents and more rigid than PDMS. PDMS device can be swollen by many organic solvents [155], which can degrade the performance of droplet generator and reduce the range of emulsions that can be produced. Moreover, PDMS devices might not be suitable for commercialization due to limited shelf life [170] and low mass production rate.

The PMMA device is reusable even when the channel has been contaminated or the junction has been blocked by foreign objects such as fiber and dust. It can be reused again and performs as new after cleaning and re-bonded with a new tape. Because of the reusability of the PMMA device, it helps during development of Automated Droplet Measurement (ADM) software, which will be discussed in chapter 4.

However, the cost involved to fabricate the molds for PMMA device is high. This involves fabrication of two molds, and the use of an industrial grade injection
molding machine. Therefore, PDMS devices are used for experimenting a new way to control bubble and droplet generation using ultrasonic transducer, which will be discussed in chapter 5 & 6.

3.4. Summary
Two methods to fabricate the microchannel in PMMA for droplet generation have been explored. Laser engraving method was ruled out as the channels produced are rough and it will impede the visibility and measurement of droplets under microscope. PMMA devices have been fabricated by injection molding successfully. The open channel of the PMMA device was enclosed by adhesive tape. The tape was chosen from different types of tapes after performing variety of test including pressure test. Droplet generation in PMMA has been evaluated due to the lack of study in the literature, especially those bonded by adhesive tape. The effect of the presence of surfactant to the generation of monodisperse droplets has been evaluated briefly. Multiphasic behavior was studied to find out the characteristic of the flow under different sets of flow rates. In the end, the capability of the fabricated PMMA has been evaluated by characterizing the generated droplets under different sets of flow rates. This study offers insight of the limitations and the merits of the fabricated PMMA devices, which is important for deciding which method and material to choose for different purposes.

Although PMMA device offers some merits over PDMS, however, PMMA device is not used for the fulfilment of the research objective that requires iterative of different channel designs. The usage of PMMA device will not only involve high fabrication cost but also longer lead time, which outweighs its own merits. Therefore, PDMS
devices are used to explore a new way to control bubble and droplet generation using ultrasonic transducer.
Chapter 4: Automated Droplet Measurement

In this chapter, the development of new video processing software for droplet measurements will be discussed. The first version of software was first written to evaluate the capability of OpenCV. It was followed by second version with improvement on performance, usability and reliability. The software eventually evolves into an Automated Droplet Measurement (ADM) system, which is fully integrated with the camera software development kit (SDK) for even better processing speed. ADM is fully automated with the object based background extraction operation (BEO) and automated binary threshold value selection (BTVS). The application of ADM is discussed at the end of the chapter to showcase the ability of ADM to perform in situ measurement with high processing speed and shorter measurement duration.

4.1. Preliminary work

4.1.1. First prototype

The first version of ADM was a simple program that recognizes the contours of droplets from a video file frame-by-frame and relate the contours from the same droplets recognized from the previous frames. Although image recognition programs, such as ImageJ, were already in existence during the time of development, they were not efficient in performing a large number of droplet measurements from a video. The prototype was developed with the aim of integration with camera SDK (software development kit) and as a feedback component to control the droplet size.
The first version was written in Visual Studio C++ 2010 Express with OpenCV package. It was built to evaluate the capability and suitability of the OpenCV package for droplet measurements. The user interface (UI) of the software was rough as it only uses the basic built-in UI elements provided by OpenCV.

Figure 4-1 shows a screenshot of the first version software, which is able to measure the basic parameters such as the length, width, area and the travelling speed of the droplet. The UI elements provide only basic function for image and data representation with little customization capability. The program has separate windows for raw video playback, tracing parameter control, measurement result and visual feedback. This version showed encouraging performance improvement over similar recognition software written in MATLAB. The tracing speed using OpenCV is always at least twice as fast.

Figure 4-1 Screenshot of prototype droplet measurement program.
4.1.2. Second prototype

Version 1 shows the potential of using OpenCV package to perform droplet measurements from video at high processing speed. In the following subsections, improvements were done to addresses the shortcomings of the first version.

A. User interface

In version 1, a user needs to modify the code in order to select desired video file for analysis. The usage of this software could be challenging for a person without much experience in programing. Therefore, there is a need to go beyond using the basic built-in UI elements provided by OpenCV.

Version 2 was written in Visual C#. Visual C# works with UI class library of .NET Framework for creating WinForms applications. The library provides access to the native Windows controls like listview, textbox, button and checkbox which are essential for enhanced UI.

Emgu CV was used as the wrapper to the OpenCV package in C# coding language. In this version, the raw video playback, tracing parameter control, measurement result and instantaneous visual feedback were integrated and consolidated within a single window. Additionally, a graph module was included to help visualizing the trend of the measured data. The program retrieves video files directly from a high speed camera (HiSpec 4, Fastec Imaging Corporation) using the camera SDK. Figure 4-2 shows a screenshot of version 2.
B. Contour linking algorithm

Figure 4-3 Droplets moving in meandering channel.

Version 2 also improves the algorithm for the linking of contour of droplets. In version 1, the linking of contours with the previous frame contours is based on a very simple algorithm. For the algorithm to work properly, the camera must be adjusted in the way that the droplets are moving parallel to y-axis and the centroid y-coordinate of the front most droplet must have the smallest value. This simple algorithm failed to trace the droplets moving in a meandering channel (Figure 4-3)
as the movements are not always parallel to y-axis. The algorithm also fails to track the droplets moving around the turns as the direction of droplets change.

This limitation was overcome by using the nearest neighbor algorithm. For every frame, the pair of contours (each from previous and current frame) with the shortest centroid distance, and within the maximum distance value is linked up. The linkage operation involves the addition of the current contour to the list of contours according to the group number. For the contours in the previous frame which are not linked up, the objects are considered to have just gone out of the region of interest and the measurement for the contours groups is regarded as completed. On the other hand, for the contours in the current frame which are not linked up, the objects are considered to have just appeared in the region of interest. For each of the newly appear object, a new group of a list of contours is created with its assigned serial number. This is to assist the link up process in the future frames.

C. **Visual feedback performance**

In version 1, visual feedback was done after processing a single frame. This includes the display of a raw video frame, the traced contours and the quantitative data. However, since the processing frame rate is usually higher than the standard video playback framerate {30 frame per second (FPS)}, the instantaneous visual feedback proved to be very strenuous to the computer resource. This causes intermittent program freezes in lower performance computers (such as Pentium 4, 1024 MB of RAM) when the visual feedback is executed at the same rate as the processing frame rate.

In version 2, the performance of the visual feedback was improved by optimizing the code for better thread management. The user interface, image processing and
visual feedback are all run under separate threads. The creation of image buffer and result data buffer allows the interaction between threads while not consuming too much computing power. A timer has been incorporated in the buffers unit to prevent it from being updated and read too frequently (<40 ms between updates).

4.1.3. Further improvement

Although version 2 was much better than version 1 in terms of performance, usability and reliability, there are still rooms for improvement. The software needs to be better than Droplet Morphometry and Velocimetry (DMV) [44] by Basu, which was newly released during the time. Firstly, the processing time could be decreased even further by better integration with camera SDK. Secondly, background subtraction should be included. The capability of version 2 was limited without background subtraction as some background features were tracked when the visibility is poor. Additionally, the program should automate background finding and threshold finding to achieve full automation of droplet measurements.

In the flowing sections, the development of an Automated Droplet Measurement (ADM) software will be described in detail. The ADM allows full automation of droplet measurements and addresses the limitations of currently available software.

4.2. Automated droplet measurement (ADM)

4.2.1. Introduction

ADM is built not only to perform droplet measurements automatically and reliably on video files but also for better integration with the camera SDK for higher processing speed. High processing speed facilitates in situ measurement, which will serve as an invaluable tool which allows fast response monitoring on the generated droplet. This function will allow users to implement immediate changes to rectify
any abnormalities detected. For example, leakages or the presence of foreign particles in the microfluidic channels will affect the flow behavior which in turn affects the size, speed and polydispersion of the generated droplets. This is also usually not apparent and obvious and can only be detected after a period of time or during routine checks. In certain cases, inconsistent results obtained after the evaluation entail repeated experiments which can be averted if in situ measurements are done promptly. However, to the author's knowledge, no such method or software exist despite the obvious need for such application.

The enhanced capability by ADM will be beneficial and useful for different droplet based applications. For example, in situ automated droplet measurements will allow tuning of the droplet to the required size accurately; by adjusting the flow condition such as flow rates of syringe pumps, either manually or automatically using a feedback loop. This is especially important for applications such as cell encapsulation [171] that require high precision control in droplet size.

4.2.2. Typical droplet measurement process

Figure 4-4 illustrates a typical process flow for droplet measurements. In brief, the process can be divided into online and offline steps. The online step happens during the capturing of droplet and transferring of the recorded video while the offline step processes the video and result which takes place after the video was captured and transferred. In the online step, the process initiates by recording droplets using a high speed video camera which is fitted onto a microscope. The recording duration depends on several factors such as the speed of the droplets, droplet production frequency and number of droplets to be evaluated. This in turn determines the frame rate to be used and the duration of the recording. In general, the frame rate
has to be higher than the droplet production frequency to allow an accurate analysis. For instance, it takes 0.1 s to record 1000 frames at 10000 frame per second (FPS) on 35 droplets generated at a production rate of 350 Hz. The recorded video is then transferred from the camera’s temporary storage to a permanent storage such as computer hard drive or external memory storage. The time taken depends on the transfer speed and the writing speed of the video data. As an example, this process takes more than 5 s to transfer 1000 frames, 520x64 pixels video data from a camera (Phantom Miro M310) without any video compression to a computer hard drive.

In the offline image evaluation, the experimental videos first need to be processed before any droplet measurements can be extracted for further analysis. This first involves extracting the background and the selection of an appropriate threshold value to convert the video into binary images. This step is usually done manually by inputting and selecting the appropriate parameters. The parameters are then checked by scanning through the video in the software to inspect if the contours are recognized properly. Next, the droplets are tracked and monitored to avoid repetition and double counting. This is done by analyzing each frame of the video.
In each frame, the background is removed and the image is converted into a binary image. The contours of the droplets are then identified from the binary image. After filtering the contours to exclude non-droplet contours (i.e. finger of stream), each of them is compared and linked to one of the contours obtained from the previous frame. The time used in this process depends on the image recognition library and the efficiency of the coding. For instance, a MATLAB based DMV video recognition software by Basu [44] takes about 45 s (Windows 7 PC with Intel Core i7 M620 CPU) to process 945 frames of video with resolution of 520x64.

Table 4.1 Difference between conventional and new process flow

<table>
<thead>
<tr>
<th></th>
<th>Conventional process flow</th>
<th>New process flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Offline process</td>
<td>Need offline process as</td>
<td>All process is done online and automatically</td>
</tr>
<tr>
<td></td>
<td>background extraction and</td>
<td></td>
</tr>
<tr>
<td></td>
<td>threshold value selection</td>
<td></td>
</tr>
<tr>
<td></td>
<td>must be done manually</td>
<td></td>
</tr>
<tr>
<td>Permanent storage</td>
<td>Need to store in a</td>
<td>Do not require permanent storage as data are</td>
</tr>
<tr>
<td></td>
<td>permanent storage for</td>
<td>extracted through direct streaming from</td>
</tr>
<tr>
<td></td>
<td>offline process</td>
<td>camera temporary storage</td>
</tr>
<tr>
<td>Automated stop</td>
<td>Not supported as the</td>
<td>Automated processing is able to count the</td>
</tr>
<tr>
<td>streaming signal</td>
<td>software does not able to</td>
<td>number of droplet directly and stop the</td>
</tr>
<tr>
<td></td>
<td>count the droplet directly</td>
<td>streaming when enough samples has been</td>
</tr>
<tr>
<td></td>
<td>and quickly from video file</td>
<td>collected</td>
</tr>
</tbody>
</table>

The study of the droplets characteristic in different flow fields or external conditions requires multiple experiments and repeated tests. Usually, tens or even hundreds of tests have to be performed before one can understand and elucidate the unique experimental observation. When coupled with the above image processing technique, it usually takes many painful months or even years before plausible conclusions are reached after the analysis. Recognizing this, it is impractical and
time consuming to perform the above process. The process is also highly inefficient as the droplet measurements can only be obtained after an offline analysis. Rapid and automated droplet measurement software will address this inadequacy. A new process flow is proposed in Figure 4-5 that allows droplet measurements to be carried out online and run automatically. The difference between the process flow is summarized in Table 4.1.

4.2.3. New process flow with ADM software

![Figure 4-5 New droplet measurement process flow with automated preprocessing step and fast tracking speed.](image)

In order for the new process flow to be efficient and practical, three main bottlenecks that hamper the rapid measurement of droplets are addressed. Two main critical functions, namely the extraction of background image and selection of threshold values, are first identified and automated. Then the tracking speed of the droplets by optimizing the coding for tracking to enable a fast detection and measurement system are addressed. The new process flow can be executed seamlessly and run online for rapid droplet measurements.

The first bottleneck is the extraction of background information. The implementation of an effective background extraction operation (BEO) must (i) suit
the characteristics of the droplet movement, and (ii) be universally applicable to different environments.

The automation of the binary threshold value selection (BTVS) operation suppresses the second bottleneck: by selecting its optimized value automatically from the operation, the prominent contours from each video frames can be properly recognized by the software.

For most video conditions, the two operations described enable the software to track the droplets reliably. Furthermore, an unmanned processing step drastically overcomes manual operation in terms of time and invariability of objective criteria for the selection of optimal parameters. Also, for most situations, finding the optimal parameters for video tracking resolve the other processing parameters such as erosion, dilation and advanced filtering measures. The two devised algorithms will be explained in detail in following section.

The third bottleneck entails the speed of ADM software to run the two mentioned automated processing operations. In addition, the software must be able to track the droplet at a much higher image processing speed than the currently available software. To do so, the target is to achieve a tracking speed comparable to or higher than the transfer speed of video data. The viability of the new process flow is thus linked to that target. The improvement provided by the new process lies on the streaming of video data into a PC memory instead of transferring it to a permanent storage. As the I/O (input/output) speed of PC memory is higher than a permanent storage, it allows simultaneous video frame streaming from camera and access by ADM for the tracking step. For BEO and BTVS operations though, the software
accesses the temporary storage directly as the number of frames is small (about 100) and the frames are picked randomly.

By implementing the new process flow with the proposed ADM, the tracking speed is now limited by the transfer/streaming speed only, as the tracking speed is comparable or faster than the transfer speed. This will further cut down the time spent on the whole process. Moreover, simultaneous streaming and tracking allows the software to stop the streaming after tracking a sufficient number of droplets, which reduces the time taken even further.

4.2.4. Automation of processing steps

A. Object based background extraction operation (BEO)

As mentioned in section 2.5.1, using a statistical survey for background extraction operation (BEO) as suggested by Basu in DMV is not ideal for all situations as it fails to extract a proper background for droplet generated at low flow rate ratio. This curbs the universality of the method to perform BEO, where the study of droplet generating at low flow rate ratio is common. As this limitation can impede the automation of the processing step, a new and more universal object based BEO that suits the characteristics of the droplets travelling in microchannel is developed.

As illustrated in Figure 4-6, an object based BEO is developed that is derived from a modified statistical method. The operation starts by generating an average image \((A1)\) using 40 randomly selected frames \((F1 – F40)\), where 40 is a number that balances BEO accuracy with operation speed and cost as described next. Then the non-background regions in \(A1\) are removed by using an operator \(h\) which extracts fragments of background regions from randomly selected frames \((F41 – F80)\). Image \(R1\) shows the result of the first iteration of the operator \(h\) on image \(A1\) with
image $F_{41}$. The area of the non-background regions in image $A_1$ is reduced after $h(A_1,F_{41},A_1)$ operation. The area is reduced further in the second iteration applying $h(R_1,F_{42},A_1)$, as shown in image $R_2$. After 40 iterations, the area of the non-background regions is reduced, as shown in image $R_{40}$. Further iterations produce exponentially decaying, negligible improvements in background extraction.

Figure 4-6 Overview of object based BEO (Background extraction operation), where $F_1$-$F_{80}$ are randomly selected frames, $A_1$ is the average of the frames while $R_1$-$R_{40}$ are the frames with reduced non-background area.

Operator $h$ is crucial for the effectiveness of the proposed object based BEO. The operation discriminates between the moving objects and the background in the randomly selected video frame. This operator determines the regions to extract from the randomly selected video frame and patches them on the average image. The result of applying $h$ on images with 256 gray intensity levels is illustrated in Figure 4-7. Transformation of image $R_1$ to $R_2$ by $h(R_1,F_{42},A_1)$ is used as an example to show how the operator works in detail.
The operation starts by performing a preliminary background removal (PBR) procedure on image $F_{42}$ using $A_1$ as an average image. The formula used for the procedure is shown in equation (4-1) & (4-2), where $F$ is the image to perform PBR while $A$ is the average image.

$$D_{1ij} = f(F_{ij}, A_{ij}) = \begin{cases} 
255, & \text{if } l_2(A_{ij}) \leq 0 \lor l_2(F_{ij}) > l_2(A_{ij}) \\
0, & \text{if } l_2(F_{ij}) < 0 \\
255 \times \frac{l_2(F_{ij})}{l_2(A_{ij})}, & \text{otherwise}
\end{cases}$$  \hspace{1cm} (4-1)

$$l_2(l_{ij}) = \frac{245(l_{ij} - I_{min})}{I_{max} - I_{min}} + 10$$  \hspace{1cm} (4-2)

$F_{ij}$, $A_{ij}$, and $l_{ij}$ are the pixel intensity matrices of the corresponding images. The $I_{min}$ and $I_{max}$ used in equation (4-2) are the minimum and maximum pixel intensity value of image $F1$ to $F40$ found out during the generation of image $A1$. $l_2$ function is
used to optimize the intensity range. Note that the minimum pixel intensity value yielded is 10 in order to avoid oversensitive division operation at single digit value of intensity. As shown in Figure 4-7, image $D_1$ is the result after performing PBR on image $F_{42}$. Background division is used instead of subtraction for the background removal procedure as it is more robust to illumination changes [172] and uneven illumination. This is important to produce consistent results under a variety of situations. Background division is also effective for the procedure that uses an imperfect background image like $A_1$.

Operation $h$ is then continued by converting image $D_1$ into a binary mask $B_1$ (see Figure 4-7). The $g$ procedure used for the conversion marks the moving objects in image $F_{42}$ as dark regions. The procedure starts by converting image $D_1$ into a binary image with 0 (dark) or 1 (bright) in pixel intensity value. The bright inner areas of the binary image are then filled up to mark out the moving objects. Afterwards, the dark areas are expanded with a scale relative to the width of their minimum bounding rectangle. The expansion is done to cover the shallow shadows of droplets that are not bounded by the initial dark areas.

The produced binary mask $B_1$ is then multiplied with image $F_{42}$ element by element to cover the moving objects in the image, as shown in image $M_1$. Conversely, image $B_2$, a complimentary binary mask of $B_1$, is multiplied with $R_1$ element by element to produce $M_2$. Afterwards, addition of $M_1$ with $M_2$ produces image $R_2$, [the result of the operator $h$ on the image vector \{$R_1, F_{42}, A_1$\}, i.e. $R_2 = h(R_1, F_{42}, A_1)$] which has a closer representation of the real background compared to $R_1$. Finally, the BEO process can be formally defined as the sum of an average operation over an image vector \{$F[I]$\}_{i=1,...,n}, where $n$ is a properly selected order.
(here, \( n = 40 \)), plus an operator \( h \) composed of \( n \) nested operators \( h(R[J - 1], F[J + n], A1) \) on the image vector \( \{F[J + n]\}_{j=1,...,n} \), starting with the average image \( R0 \equiv A1 \).

**B. Automated binary threshold value selection (BTVS)**

As mentioned in section 2.5.2, the available DMV requires the user to perform BTVS manually. This is done by changing the value of binary threshold and checking the converted binary image. As the selection is done visually, the selected value is different not only from one user to the other but also from time to time within the same user. In order to resolve the issue, a method to perform BTVS automatically has been developed. The method enables automatic selection of an optimum threshold value with high repeatability.

Automated BTVS starts by selecting one frame from a video to produce multiple binary images converted using different threshold values. As the frame is an image with gray intensity level from 0-255, the frame is converted into 254 binary images using threshold values from 1-254 in one operation of automated BTVS. Afterwards, contour recognition is done on each binary image, ascending from the binary image converted at the lowest threshold value (\( T = 1 \)) to the highest threshold value (\( T = 254 \)). The contours recognized at each threshold value are grouped together according to their centroids using a nearest neighbor search. By the end of the nearest neighbor search at \( T = 254 \), each group will contain at least one or multiple contours with similar centroid, collected at subsequent threshold values.

Table 4.2 shows an example that illustrates the process of the automated BTVS on the droplet image in the table. The outline of the droplet first appears at binary
image $T = 12$ as two dots. The outline becomes more prominent when the threshold value increases, as shown in the binary images in Table 4.2. After performing filtering of contours and nearest neighbor search, there is only one group of contours with similar centroids and recognized from binary images converted with increasing threshold value ($T = 102$ to $T = 252$). The recognized contours from binary image $T = 101$ and below are unqualified either for not reaching the minimum area (e.g. small contours in $T = 12$ & $T = 70$) or with circularity ($4\pi \times \frac{\text{Area}}{\text{Perimeter}^2}$) lesser than 0.5 (e.g. disconnected contours in $T = 70$ & $T = 101$). The recognized contours from binary image $T = 253$ and $T = 254$ are also unqualified as the spiky contours have low circularity.

**Table 4.2 The process data of automated BTVS on a droplet image**

<table>
<thead>
<tr>
<th>(Droplet image)</th>
<th>Threshold value</th>
<th>Binary image</th>
<th>Recognized contour</th>
<th>Circularity</th>
<th>Qualified</th>
</tr>
</thead>
<tbody>
<tr>
<td>First appearance</td>
<td>12</td>
<td></td>
<td></td>
<td>NA</td>
<td>No</td>
</tr>
<tr>
<td>Under</td>
<td>70</td>
<td></td>
<td></td>
<td>NA</td>
<td>No</td>
</tr>
<tr>
<td>Just under</td>
<td>101</td>
<td></td>
<td></td>
<td>0.0185</td>
<td>No</td>
</tr>
<tr>
<td>Min</td>
<td>102</td>
<td></td>
<td></td>
<td>0.8836</td>
<td>Yes</td>
</tr>
<tr>
<td>Median</td>
<td>177</td>
<td></td>
<td></td>
<td>0.8621</td>
<td>Yes</td>
</tr>
<tr>
<td>Max</td>
<td>252</td>
<td></td>
<td></td>
<td>0.6754</td>
<td>Yes</td>
</tr>
<tr>
<td>Just over</td>
<td>253</td>
<td></td>
<td></td>
<td>0.3203</td>
<td>No</td>
</tr>
</tbody>
</table>

After sorting the filtered contours into groups, the range of threshold value is known from each group with features recognizable as qualified contours. In situations where there is only one group of contours, the automated BTVS will select the median of the range as the suitable threshold value. For the example in Table 4.2,
the outline of the droplet image is recognizable in the range from $T = 102$ to 252. Thus, the median value, 177, will be selected.

In order to increase the repeatability of the automated BTVS, more than a droplet image needs to be sampled. The policy, outlined in the following, will be subsequently discussed in detail in section 4.3.2. By increasing the sample size, there will be more groups of qualified contours. Furthermore, as more frames are sampled in the automated BTVS, it is unavoidable to have groups of contours that have small range of threshold value, representing the non-prominent features such as a faint background object. The groups of this kind can be excluded by setting a minimum acceptable range, such as 20 set in ADM software. Finally a suitable threshold value can be selected from the remaining groups by analyzing the range of threshold values of each group. The top 50% groups of the larger range are selected and the medians of their range are collected. The final value will be the median of the collected medians. The lower 50% groups are excluded from the collection as they may contain groups of shorter range recognized from the droplets near the edge or the extended fingers right before separating from the dispersed phase.

4.2.5. Integration of ADM and camera SDK
After implementing object based BEO and automated BTVS in ADM software, the processing step has been made fully automated, together with the tracking operation. The implementation also ensures proper tracking of the moving object by the ADM software according to the characteristic of the video, under most of the situations. The whole measurement process flow can then be computerized by developing an integrated program. As shown in Figure 4-8, the two main modules used in the program are the Software Development Kit (SDK) provided by camera
manufacturer and ADM software. The former module is used to control the high speed camera and retrieve the video data from it while the latter is to perform the processing step automatically. By using the integrated program, ADM is able to access the video frames directly from the camera through the SDK. This eliminates the lengthy video file transfer process that involves permanent storage on a PC.

<table>
<thead>
<tr>
<th>Integrated program</th>
<th>ADM software (C#)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thread control</td>
<td>BEO</td>
</tr>
<tr>
<td>Resource management</td>
<td>BTVF</td>
</tr>
<tr>
<td>Camera SDK (C#)</td>
<td>Tracking</td>
</tr>
<tr>
<td>Camera control</td>
<td>EMGU CV (C#)</td>
</tr>
<tr>
<td>Frame reading</td>
<td>Image processing</td>
</tr>
<tr>
<td>Frame streaming</td>
<td>Presentation</td>
</tr>
<tr>
<td></td>
<td>Windows GUI (C#)</td>
</tr>
<tr>
<td></td>
<td>ZedGraph (C#)</td>
</tr>
<tr>
<td></td>
<td>OpenCV (C/C++)</td>
</tr>
</tbody>
</table>

**Figure 4-8 The simplified architecture of the program integrating camera SDK and ADM software.**

ADM software was written in Visual C# using OpenCV through Emgu CV, a wrapper to the OpenCV library. Visual C# is adopted for efficient Windows Graphical User Interface (GUI) development and it will help to design instantaneous result feedback without consuming large computer resources. The coding is optimized to match or surpass the video data transfer speed. The simplified architecture of the integrated software is illustrated in Figure 4-8. Thread control is required for program features such as simultaneous streaming & tracking and instantaneous visual feedback. Resource management is particularly important for intensive processes such as frame streaming and tracking. Some of the results are represented by graph plotting using ZedGraph, an open source class library and user control for drawing 2D Line, bar and pie charts.
4.3. Discussion

4.3.1. Object based BEO

A. Choosing a suitable method for PBR procedure

As mentioned in the earlier section, operation $h$ is crucial for the effectiveness of object based BEO. The operation is made possible by having an effective PBR procedure using an average image, which is an imperfect background. The result of the procedure has been shown in Figure 4-7, where the background in image $F42$ is removed using image $A1$ to produce image $D1$ under the $f(F42,A1)$ procedure. Other methods have also been tried for the PBR procedure before adopting method $f$ stated in equation (4-1) & (4-2) for the procedure. The methods are $r_1$, $r_2$ & $r_3$, which are shown in equation (4-3). For the symbols used in the equations, $F_{ij}$ is the image to perform PBR, $A_{ij}$ is the average image, $l_1$ is a leveling function to optimize the range of intensity value, while $I_{min}$ and $I_{max}$ are the minimum and maximum intensity value, respectively. $I_{min}$ and $I_{max}$ are surveyed during the generation of the average image.

\[
\begin{align*}
    r_1(F_{ij}, A_{ij}) &= 128 + 0.5l_1(F_{ij}) - 0.5l_1(A_{ij}) \\
    r_2(F_{ij}, A_{ij}) &= 255 - |l_1(F_{ij}) - l_1(A_{ij})| \\
    r_3(F_{ij}, A_{ij}) &= \begin{cases} 
    255, & l_1(A_{ij}) - l_1(F_{ij}) < 0 \\
    255 - (l_1(A_{ij}) - l_1(F_{ij})), & \text{otherwise} 
\end{cases} \\
    l_1(I_{ij}) &= \frac{255(I_{ij} - I_{min})}{I_{max} - I_{min}}
\end{align*}
\]

Method $r_1$ is a standard background subtraction. The pixel in image $r_1(F,A)$ takes the middle value (128) when $F_{ij} = A_{ij}$. The pixel intensity is darker ($<128$) when
$F_{ij} < A_{ij}$ while lighter ($> 128$) when $F_{ij} > A_{ij}$. Method $r_2$ is an absolute background subtraction. The pixel in image $r_2(F,A)$ is 255 when $F_{ij} = A_{ij}$. The pixel intensity becomes darker ($< 255$) whenever there is a difference between $F_{ij}$ and $A_{ij}$. Method $r_3$ is similar to $r_2$, but the pixel intensity remains 255 when $F_{ij} > A_{ij}$.

As shown in Figure 4-9, all the methods are able to remove the outlines of the channel wall effectively. Interestingly, the images produced by method $r_3$ and $f$ do not have the shadowy droplet outline shown in image inherited from image $A$. This is because the methods do not discriminate the change for $F_{ij} > A_{ij}$. Method $r_3$ and $f$ are especially suitable for capturing droplets because the droplets outlines are darker than the background. Method $f$ is preferred than method $r_3$, as the images produced using $f$ method shows the droplets outlines more clearly and in higher contrast. Additionally, method $f$ is insensitive to the lightning distribution, as exemplified in case (b) of Figure 4-9. For this case, the outline of droplet in the darker region is much clearer from the image produced using method $f$ than the one using method $r_3$. 

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After performing the assessment on several other videos using the above stated methods, method $f$ is chosen as the PBR procedure in BEO. Method $f$ is able to produce consistent result in variety of lightning condition, with high contrast images showing the droplets outlines clearly. This allows the use of a fixed threshold value, independent of the video condition, in $g$ procedure to produce a binary mask for operator $h$.

**B. Application of BEO on different videos**

The proposed object based BEO has been tested on different types of video. As shown in Figure 4-10, the videos include (a) droplet splitting, (b) droplet formation, (c) droplets travelling under channel with a variety light distributions and (d) bubble formation. According to the test results, object based BEO is able to extract the correct background for droplets/bubbles captured under different situations. The test result is also compared to mode BEO (a statistical method, by setting the intensity values to their modes), which highlights benefits of object based BEO.
Mode BEO fails to generate the correct background for from video (a), (c) and (d), as some of the intensity values are from the moving objects. This generates the unwanted darker or lighter tails in the extracted backgrounds.

![Figure 4-10 BEO on different types of video.](image)

<table>
<thead>
<tr>
<th>Video</th>
<th>(a)</th>
<th>(b)</th>
<th>(c)</th>
<th>(d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Object based BEO</td>
<td>![image]</td>
<td>![image]</td>
<td>![image]</td>
<td>![image]</td>
</tr>
<tr>
<td>Mode BEO</td>
<td>![image]</td>
<td>![image]</td>
<td>![image]</td>
<td>![image]</td>
</tr>
</tbody>
</table>

4.3.2. Automated BTVS

A. Application of automated BTVS

Figure 4-11 shows the result of the automated BTVS operation applied on a video capturing the droplet formation. The video was the same as the one used in case (b) in Figure 4-10. The operation was done on an image combined by 5 frames from the video. Each frame was selected randomly from the video and performed with background removal before the combination. There are 14 groups of qualified contours recognized from the image under the operation. Each group was labeled in the figure with a pair of rectangles in red and blue. The red rectangle was the bounding rectangle for the qualified contour recognized at the lowest binary threshold value from a group, while the blue one is at the highest value. For instance, object (2) was recognizable as a qualified contour from the image starting from threshold value of 100 until 253, with range of 154.
Table 4.3 lists the groups recognized from the video using the automated BTVS. As all the sizes of the range are larger than 20, none of the groups are removed from the list. Each group is then ranked according to the size of range, from the biggest to the smallest. Afterwards, the top 50% groups in terms of the size of range will be included in the selection of a suitable threshold value. For this case, group (3), (4), (6), (7), (8), (10) and (14) are excluded. Object (7) has the smallest in range as it is recognized from the extended finger right before separating from the dispersed phase. Object (14) is the next smallest recognized from a droplet near the edge of the frame.
Table 4.3 Data of the groups of recognized contours. The median is collected only from the top 50% of the group in terms of size of range. Therefore, only the median for the rank 7 or higher groups are found out. The medians of the included groups are then collected. Finally, by computing the median of the collected medians, automated BTVS selects value 176 for the threshold value. In order to evaluate the quality of the selected threshold value, the object images and their converted binary image at the selected threshold value are compared. The object images are listed in Table 4.3, showing binary images and recognized contours according to their associated groups. The binary images generated at the selected threshold value (176) are found to represent the object outlines correctly. Additionally, the recognized contours from the binary images are
able to trace the outlines of the objects closely. Among the contours generated at T=176, contour from group (7) is unqualified automatically as it is recognized from an object with a broken outline. The contour has circularity value lower than 0.5. The exclusion of the group (7) at T=176 is consistent to the intended result as it is not yet a droplet.

B. **Repeatability of automated BTVS**

![Histogram of the obtained threshold value in 100 times using automated BTVS algorithm.](image)

As the automated BTVS operation is sampled from image combining 5 randomly selected frames from a video, the determined threshold value can fluctuate from one execution of the operator to the other. Here, the fluctuation of the value and its implication to the measured droplet area is studied on the same video. Figure 4-12 shows the histogram of the threshold value obtained using the automated BTVS for 100 times. The threshold value fluctuates between 174 and 178. The measured droplet area changes slightly from one threshold value to the other, from 1133.3 to 1136.1 pixel². For this case, the standard deviation of the measured area is 0.7427
pixel², or with CV of 0.0655%. This value is small enough to ensure high repeatability for the measurement.

4.4. Application of software
4.4.1. Droplet generation
ADM software has been used to perform droplet measurement on a typical droplet generation video. The PDMS droplet generation device used here has a channel width and height of 100 µm and 45 µm respectively. The dispersed phase was deionized water while the continuous phase was mineral oil (M5904, Sigma Aldrich) with 5%w/w surfactant (Span 80, Sigma Aldrich). The volumetric flow rates were maintained using syringe pumps (neMESYS, Cetoni) at 200 µL/hr for the dispersed phase and 1000 µL/hr for the continuous phase. After measuring 30 droplets, the average area, speed and perimeter were found to be 4503.1 µm², 100.51 mm/s and 255.7 µm respectively. The coefficient of variations (CVs) for the three values are all lesser than 0.2%

Figure 4-13 shows a screenshot of ADM software. The software is able to trace each of the recognized contours, grouped accordingly using nearest neighbor finding, after tracking of droplets. For the plotting of data, common parameters needed in droplet measurements are listed. For the plots, (a) is time against group ID, illustrating the time and duration of the occurrence of each droplet, (b) is y position against x position, which shows the imperfectness of the stage alignment and also highlights the deviation of droplet movement from the centerline due to some defects in the channel, (c) is contour perimeter against time, showing the trend of the dimension across the time (d) is a histogram showing the distribution of top-view droplet areas.
Figure 4-13 Screenshot of ADM software with data plots: (a) time against group ID (b) y position against x position (c) contour perimeter against time (d) histogram of droplet areas.

A. Comparing ADM result with volume flow rate

The accuracy of the measurement was checked by comparing the deduced flow rate from the measurement data to the set flow rate at the pump. The flow rate was deduced using equation (4-4), together with the estimated droplet volume equation, equation (4-5), for droplet in a channel with rectangular cross section [173].

\[ Q = fV \]  \hspace{1cm} (4-4)

\[ V = hA - 2p\left(\frac{h}{2}\right)^2\left(1 - \frac{\pi}{4}\right) \]  \hspace{1cm} (4-5)

The \( Q \), \( f \), and \( V \) values are the deduced flow rate, droplet generation frequency and droplet volume respectively, while \( h \), \( A \) and \( p \) are the height of channel, top-view area and perimeter respectively. As the measured droplet generation frequency is 353.7 Hz, the corresponding deduced flow rate is 187.3 µL/hr, is on the order of the
set flow rate (200 µL/hr) at the pump for the dispersed phase. The deviation can be attributed to the volume equation used, which may need appropriate correction factors to account for the roundness features in the direction normal to the plane of view in this case. Other factors such as inaccuracy in the measurement of channel height, recognition of contour, and syringe pump may also contribute to the deviation. Here, the length-average error is only 2.17% (cube root of the ratio of the deduced and the set volume flow rate), which is an acceptable value.

B. Comparing ADM result with DMV result
DMV has also been used to measure the droplets from the same droplet generation video to compare its result with ADM result. The measurement by DMV was done without background subtraction, with threshold parameter value of 0.52. The average area, average speed and production frequency are 4729.9 µm², 101 mm/s and 353.7 Hz respectively. These values are comparable to the result obtained using ADM.

4.4.2. Droplet splitting
ADM software has also been used to perform measurements on droplet splitting. The device used in this video was a PMMA device fabricated by injection molding [162]. The channel width and height were both 200 µm. The dispersed phase was deionized water while the continuous phase was light mineral oil (330779, Sigma Aldrich) with 2%w/w surfactant (Span 80, Sigma Aldrich). The volumetric flow rates were maintained using syringe pumps at 35 µL/min for the dispersed phase while 100 µL/min for the continuous phase. The frames of the video are shown in image F41 and F42 of Figure 4-6.
ADM software has a special image box for visual feedback in order to monitor the tracking effectively. This feature is also suitable for the splitting case. The software updates the special image box with a representative contour from its contours group once the group exits from the visible region. The representative contour is the contour at the half of the group’s visible period. For example, contour at frame 20 is chosen from a group appearing from frame 11-29. The contour is then filled with the color according to the group number and drawn on top of the image box, as shown in Figure 4-14(a). As the image box is not cleared for each draw, some remnant contours can still be seen around the 3 filled contours. This feature helps to have a snapshot of the history of tracking. Erroneous tracking due to the factors such as selection of wrong binary threshold value can be easily spotted as the filled contours are scattered around the channel, as shown in Figure 4-14(b).

![Figure 4-14 The result of droplet splitting measurement taken from screenshot of the ADM software (a) visual feedback for correct tracking (b) visual feedback for erroneous tracking at low binary threshold value (c) y position against x position of centroids (d) droplet movement orientation against x position (d) changing of droplet area against x position (e) histogram of droplet area.](image)

Figure 4-14 also includes some useful plots generated instantaneously from the ADM software. Plot (c) tracks the centroids of all contours with their according to their group color. This is to show the contours are traced and grouped properly.
during the tracking process. Plot (d) shows the orientation of droplet movement against x position. The orientation of two newly spitted droplets differ the most. They return to near 0\(^\circ\) gradually as they move along the x direction. Plot (e) shows the changing of the top-view area of droplets. The area increases before the splitting and drop significantly after the splitting. Plot (f) shows the histogram of the top-view droplet areas. The count for the smaller area is 108 while the bigger area is 55. By counting the last two split droplets excluded from the data as they are still in the viewing region, the count for the smaller area is 110, which makes it exactly twice the count of the bigger area.

### 4.4.3. Speed

The integrated program is run on a PC with Intel Core i7 CPU (M620) on Windows 7. The time taken for the program to execute the object based BEO on droplet generation video (520x64 pixels, Lagarith lossless video codec) is about 0.5 s, while the automated BTVS takes about 1.1 s. For the tracking step, the program takes about 2.9 s to process 945 frames of the video, with visual feedback showing the tracking process and instant data plotting. On the other hand, DMV takes about 45 s to process the same video without visual feedback. When the visual feedback is enabled, the time taken increases to about 380 s. Therefore, ADM software is able to process the video more than 15 times faster than the MATLAB based DMV program, even when the ADM is enabled with the visual feedback and data plotting for the tracking of the droplets.

As the tracking speed (~326 FPS) is much faster than the streaming speed from camera (Phantom Miro M310) (~150 FPS) at the given resolution, the ADM software has been combined with the SDK of the camera to perform tracking operation while
streaming from the camera. This enables rapid tracking of droplet using the new process flow proposed in Figure 4-5. However, the software is still able to operate separately without the SDK using the conventional process flow by loading avi format video files.

Table 4.4 shows the time taken to perform different sets of tasks for droplet measurement. Each of the 2 videos is given 3 different sets of tasks that emulate the possible strategies to be adopted for measurement of droplets. The first 2 sets perform only the record and transfer steps online. Processing step (BEO, BTVS and tracking) is done offline after transferring the videos, as stated in the conventional process flow. The former uses DMV software to do processing step while the latter uses ADM software. Set 3 uses the new process flow to perform all the steps of droplet measurement online, by making streaming and tracking to work simultaneously.

Table 4.4 Time spent for different sets of tasks emulating the possible strategies to be adopted for measurement of droplets. The total times with red color are done with DMV while the blue color times are done with ADM

<table>
<thead>
<tr>
<th>Video</th>
<th>Droplet generation</th>
<th>Droplet splitting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Set</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Online measurement</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Process flow</td>
<td>Old</td>
<td>Old</td>
</tr>
<tr>
<td>Software</td>
<td>DMV</td>
<td>ADM</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Duration (S)</th>
<th>Record</th>
<th>Transfer</th>
<th>BEO</th>
<th>BTVS</th>
<th>Tracking (&amp; streaming)</th>
<th>Total time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1</td>
<td>6.3</td>
<td>~2.0</td>
<td>~8.0</td>
<td>44.7</td>
<td>~61.1</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>6.3</td>
<td>0.5</td>
<td>1.1</td>
<td>2.9</td>
<td>10.9</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>6.3</td>
<td>~1.1</td>
<td>1.2</td>
<td>6.8</td>
<td>9.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>~7.0</td>
<td>1.1</td>
<td>71.6</td>
<td>~100.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.6</td>
<td>1.1</td>
<td>3.6</td>
<td>18.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.4</td>
<td>1.1</td>
<td>13.8</td>
<td>16.7</td>
</tr>
</tbody>
</table>

It is obvious from the result that set 1 requires the longest time to finish both videos. The tracking step consumes the most time. For set 1, the time taken for the BEO and BTVS are only estimated as they are done manually and depends on the experience
of the operator. It is assumed that more time will be taken in the BEO for the second video as the operator needs more time to choose the other suitable background extraction scheme, after failing to extract the background using the default mode scheme.

The second set saves a lot of time for both videos compared to set 1. This is because the BEO and BTVS can be done automatically and the tracking operation is much faster. Set 3 consumes the least time. However, as the BEO & BTVS are taking the video frames directly from the camera, the times are extended for those operations compared to set 2. Furthermore, the tracking time is limited by the streaming speed from the camera. The streaming speed is slightly slower than the transfer speed in the first two sets, as it takes some time to initiate the buffer for the streaming. Overall, the time taken in set 3 is still quicker than set 2, and comparable to the transfer speed.

4.4.4. Additional features

Apart from speed improvement, ADM also comes with a batch processing feature. This feature is especially helpful for automated mass measurements of droplets. The batch processing function processes each of the video files in a list automatically. ADM also measures more droplets parameters than DMV. Beside the droplet parameters in DMV, ADM measures an additional 3 important parameters. The additional parameters are advancing/receding angle and droplet deformation which are critical for understanding the physical characteristic of droplets. These parameters are also useful to characterize and understand the droplet behavior in different conditions. Furthermore, ADM has an advanced option which allows users to modify the processing parameters to tailor to users’ needs. The contour history
review for spotting unusual tracking phenomena is also included to allow users to check for abnormalities. The important features of ADM are summarized in Table 4.5.

Table 4.5 The important features of ADM

<table>
<thead>
<tr>
<th>Feature</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed</td>
<td>More than 15 times faster than the MATLAB based DMV program. ~3000 frames in 10 seconds (520x64 pixels, Lagarith lossless video codec)</td>
</tr>
<tr>
<td>Batch processing</td>
<td>Automated processing from a list of video files.</td>
</tr>
<tr>
<td>Droplet parameters</td>
<td>A total of 24 parameters including 3 new features: advancing/receding angle and droplet deformation</td>
</tr>
<tr>
<td>Advanced option</td>
<td>User defined image processing parameters</td>
</tr>
<tr>
<td>Contour history view</td>
<td>Review tracked objects and spotting abnormalities.</td>
</tr>
</tbody>
</table>

Additionally, different from DMV, which is only available to selected requests, ADM is available freely from the ADM website (http://a-d-m.weebly.com). Tutorial and guide are also available to provide a clear and concise usage on the software. The software can also be used as a standalone without the camera SDK.

4.5. Summary
The main bottlenecks hampering the currently available software to perform in situ vital, rapid and automatic measurement on droplet based microfluidics have been identified and successfully addressed. First, the processing step has been automated, and second, the droplet measurement software has been redesigned to generate automatic, real time output at a speed overcoming that of video transfer.

Automated processing step was achieved using a newly developed object based background extraction operation (BEO) and an automated binary threshold value selection (BTVS) operation. The new object based BEO is found to be more effective,
adaptive and general than current BEO in extracting the correct background from traveling droplet video. Automated BTVS on the other hand, is able to adaptively select a near optimum threshold value that allows close tracking of droplets outlines.

Automated droplet measurement (ADM) software was developed based on the OpenCV image processing library that has much higher throughput than currently available software. The process speed (~300-420 FPS) is higher than the transfer speed (~110-150 FPS) even when the visual feedback is enabled.

Subsequently, to shorten the total time taken on droplet measurement even further, the newly developed ADM software and camera SDK are integrated together in a new process flow. This is done by performing video transfer/streaming simultaneously with video processing.

The total time for the droplet measurement using the integrated software is significantly shorter than using the old process flow with DMV software (9.2 s vs ~61.1 s, 16.7 s vs ~100.1 s). The process flow timing is even comparable to the ones without droplet measurement (6.4 s, 13.5 s). The ADM software has been publicly released for free access. The software can be used on an avi video file, without the need to integrate with the camera SDK.
Chapter 5: Bubble generation with acoustofluidic control

This chapter begins with the description of the ultrasonic transducer setup, the selection of ultrasonic transducer, the building of amplifier for the transducer, and the placement of the transducer for reliable evaluation. This is then followed chronologically by the channel design, the fluid flow setup for bubble generation and the discussion on the results and the explanation on the effect of acoustofluidic control on bubble generation.

5.1. Ultrasonic transducer setup

5.1.1. Transducer

The ultrasonic transducer chosen for the evaluation is an enclosed type. This type of transducer allows the application of ultrasonic gel for better coupling between different surfaces. This ensures efficient transmission of acoustic energy to the interface. The chosen ultrasonic transducer (328ET250, Prowave) is low cost and available off-the-shelf. It has been used for a variety of applications such as liquid level measurement, object detection and remote control. Table 5.1 shows the photo and the specifications of the transducer.

5.1.2. Custom-build amplifier for transducer

Although the transducer can be driven directly using a function generator, the peak-to-peak voltage \( V_{pp} \) from commonly available function generators is only up to 20 \( V_{pp} \). This is lower than the maximum of the selected transducer (328ET250, Prowave), which can be driven continuously at 20 \( V_{rms} \) (56.6 \( V_{pp} \)). In order to have greater ultrasonic effect and stronger vibration at the interface, an amplifier is
needed to increase the driving voltage from the function generator (33520A, Agilent).

Table 5.1 Photo and specifications of ultrasonic transducer

<table>
<thead>
<tr>
<th>Specification</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Center frequency</td>
<td>32.8 ± 1.0 kHz</td>
</tr>
<tr>
<td>Bandwidth (-6 dB)</td>
<td>1.0 kHz</td>
</tr>
<tr>
<td>Transmitting sound pressure level</td>
<td>113 dB</td>
</tr>
<tr>
<td>(at Fc; 0dB re 0.0002 μbar per 10 V&lt;sub&gt;rms&lt;/sub&gt; at 30cm)</td>
<td></td>
</tr>
<tr>
<td>Capacitance (at 1kHz ±20%)</td>
<td>2400 pF</td>
</tr>
<tr>
<td>Maximum driving voltage</td>
<td>20 V&lt;sub&gt;rms&lt;/sub&gt;</td>
</tr>
<tr>
<td>Total beam angle (-6dB)</td>
<td>33° typical</td>
</tr>
<tr>
<td>Operation temperature</td>
<td>-30 to 70°C</td>
</tr>
<tr>
<td>Storage temperature</td>
<td>-40 to 80°C</td>
</tr>
</tbody>
</table>

In order to amplify the driving voltage reliably, a high-frequency amplifier is needed. It should be capable of amplifying the driving voltage to 40 V<sub>pp</sub> in sine wave profile at least until 100 kHz. This will enable the evaluation of the response of bubble generation for a wide range of ultrasonic frequency from the transducer.

Customization is necessary as it allows building an amplifier to provide a more accurate control and adhere to the specification of the transducer. The required current to drive the transducer is very low, about 50 mA at 20 V<sub>rms</sub> (minimum impedance around the resonance frequency of the transducer is about 400 Ω). This enables the building of a suitable amplifier using a commonly available low cost op-amp.

An amplifier was built using a high-voltage, high-current op-amp (OPA552, Texas Instruments). The amplifier has slew rate of 24 V/μs and bandwidth of 12 MHz. The maximum supply voltage for the op-amp is ±30 V while the maximum continuous output current is 200 mA.
Figure 5-1(a) shows the schematic of the amplifier integrated with the selected op-amp. The elements in the schematic are arranged in a layout of a board, as shown in Figure 5-1(b), in order to facilitate the fabrication. The fabricated board with connectors is shown in Figure 5-1(c). Theoretically, the maximum gain of the amplifier is 99.1, according to the specifications of the amplifier shown in Table 5.2.

Figure 5-1 Self-build amplifier (a) schematic (b) board configuration (c) fabricated board.
Table 5.2 Specification of self-build amplifier for the selected ultrasonic transducer

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capacitors C1, C3</td>
<td>10 μF</td>
</tr>
<tr>
<td>Capacitors C2, C4</td>
<td>0.1 μF</td>
</tr>
<tr>
<td>Resistor R1</td>
<td>0.969 kΩ</td>
</tr>
<tr>
<td>Resistor R2</td>
<td>1.20 Ω to 96.0 kΩ</td>
</tr>
<tr>
<td>Gain</td>
<td>1.24 – 99.1</td>
</tr>
</tbody>
</table>

The amplifier was connected to a power supply unit (Instek GW GPC-3030D) that has two adjustable channels. Channel 1 was used to provide DC to V+ of the amplifier while channel 2 to V-. Both channels were able to provide steady DC up to 29 V. The wave quality of the amplified driving voltage (sine wave) was checked using an oscilloscope (Tektronix TDS 220). The profiles of the output sine wave voltage were maintained up to about 400 kHz before deteriorating into triangular wave.

Figure 5-2 Output voltages of amplifier for ac sine input voltages at frequency of (a) 128 kHz (b) 290 kHz.

Figure 5-2 shows the amplification of the sine wave input voltage (from the function generator) with a variety of peak-to-peak voltages when R2 is adjusted to the maximum resistance. Figure 5-2(a) shows the output voltages by the amplifier for 128 kHz input voltages. The amplifier is able to amplify the input voltage at a near constant gain of about 94.7 until it reaches the maximum allowable output voltage.
of the amplifier. The gain is acceptable as it is near to the theoretical maximum gain of 99.1.

Figure 5-2(b) shows the output voltages by the amplifier for 290 kHz input voltages. The amplifier is able to amplify the input voltage at a near constant gain until about 30 $V_{pp}$. The gain starts to drop afterwards. Instead of reaching 58 $V_{pp}$ for 128 kHz voltage input at 0.62 $V_{pp}$ (gain around 94), the voltage reaches only until 35 $V_{pp}$ for 290 kHz voltage input at 0.62 $V_{pp}$ (gain around 56). This is because the slew rate of the op-amp is not high enough to amplify high frequency with high voltage amplitude.

Figure 5-3 illustrates the differences in the amplified voltage at two different frequencies for (a) $V_{input} = 0.41 \ V_{pp}$, and (b) $V_{input} = 0.62 \ V_{pp}$, observed from the oscilloscope. For case (a), the amplitude of the amplified voltage at 290 kHz is slightly lower than the one at 128 kHz. This is because high slew rate is required for 290 kHz around the starting point and the middle point of the period, but the op-amp has difficulty to produce it. Nevertheless, the wave profile of the voltage at 290 kHz still closely resembles a typical sine wave. However, when the $V_{input}$ is increased to 0.62 $V_{pp}$, the amplitude at 290 kHz is much lower than the one at 128 kHz, as shown in case (b). Furthermore, the wave profile at 290 kHz is more triangular, due to the slew rate of the op-amp not being fast enough for most parts of the period to reach high amplitude at such a high frequency.
Figure 5-3 Output voltage in a period for sine input voltage with amplitude of (a) 0.41 V$_{pp}$ (b) 0.62 V$_{pp}$.

Figure 5-4 Output voltage amplitude for input voltage of constant amplitude (0.41 V$_{pp}$) at variety of frequencies.

Figure 5-4 shows the output voltages for input voltage at variety of frequencies (from 1 kHz to 400 kHz) under constant input voltage of 0.41 V$_{pp}$. The gain starts to drop significantly after 200 kHz. The frequency did not continued beyond 400 kHz as the sine wave becomes triangular wave when the gain becomes smaller. The
Characterization allows the capability and the limitation of the system to be known. Evaluation can be conducted after building the amplifier with known characteristic.

5.1.3. Placement of transducer
The transducer (328ET250, Prowave) was first secured beside a PDMS device, as shown in Figure 5-5. The transmitting surface of the transducer was coated with a thin layer of ultrasonic gel (ZGF, General Electric) before being attached to the glass slide. This was to ensure optimal transmission of ultrasonic energy. The distance from the center of the transducer to the center of the microchannels cross-junction was about 30 mm.

![Figure 5-5 Transducer at the side of PDMS device.](image)

Using this setup, vibration of gas-water interface at resonant frequencies was observed. However, the vibrations were very weak even when the transducer was powered at the maximum voltage (56.6 $V_{pp}$) recommended by the manufacturer. There was no change in bubble size before and after activation of the transducer.

In order to increase the intensity of the vibration, the transducer was placed directly under the device. The center of the transducer was aligned with the center of the microchannels cross-junction. The new setup is illustrated in Figure 5-6.
Figure 5-6 Transducer at the bottom of PDMS device.

Usually, transmitted light microscopy is used to observe microfluidic devices. This type of microscopy allows the capture of droplet generation with high image contrast. However, for this setup, reflected light microscopy was used to observe the channels in the PDMS device as transmitted light microscopy blocked the transmission of light through the PDMS device. Initially, difficulty was encountered in capturing a clear image of microchannels with this setup using a high speed camera (Miro M310, Phantom) with reflected light microscopy (BX51, Olympus). Moreover, high intensity light was required to acquire the image with sufficient brightness level. This is because the transmitting surface of the transducer is not smooth and therefore absorbing the light.

In order to resolve the issues, various attempts were carried out. The first was to sandwich an aluminum foil between the transducer and the glass slide. Both surfaces of the aluminum foil were coated with a thin layer of ultrasound gel before insertion. Although the image was much clearer and did not require high intensity light, this setup was unable to vibrate the gas-liquid interface. This is because most of the ultrasonic energy from the transducer has been deflected after passing through extra layers of mismatched mediums.
In the next attempt, the transmitting surface of the transducer was coated with a thin white paint. Although the paint smoothed the surface, even more light was required to capture the image with a sufficient brightness level. Eventually, microscopy with acceptable image quality was obtained while not using high intensity light. This was done by polishing the transmitting surface of the transducer with increasing grit number from 600, 800, 1000, 1500. The surface was smoothed further with a polishing compound (Premium grade polishing compound, Turtle Wax). The finished surface is now suitable for reflected light microscopy as it is flattened and reflected sufficient amount of light back to the PDMS device.

5.1.4. Power input to transducer
An alternating current (AC) source supplied the signal to the transducer. The signal was produced by amplifying a sinusoidal voltage from a signal generator (33520A, Agilent) using the self-built amplifier. An AC frequency of up to 400 kHz and a peak-to-peak voltage of up to 40 V were used in the experiment.

5.2. Channel design and fluid flow setup
The PDMS device with a cross-junction design (Figure 5-7, width w of 100 μm and height h of 43 μm) was fabricated using standard soft lithography techniques [6], which is described in the appendix. As shown in Figure 5-8, bubbles in oil are formed by flowing nitrogen gas to the central channel of the cross junction and mineral oil (M5904, Sigma Aldrich; viscosity η = 30 mPa s; surface tension γ = 33 mN m⁻¹) to the two side channels. The hydrophobic wetting nature of the PDMS material only allows stable formation of bubbles in oil for this case [174]. A syringe pump (neMESYS, Cetoni) was used to provide a constant volumetric flow rate for the oil
while a pressure controller (PPC4, Fluke) was used to maintain the pressure of the gas.

![Channel design of the PDMS device.](image)

**Figure 5-7** Channel design of the PDMS device.

![PDMS bubble generation device with an ultrasonic transducer.](image)

**Figure 5-8** PDMS bubble generation device with an ultrasonic transducer. The schematic is not drawn to scale.

### 5.3. Optical setup and others

The bubble generation was captured using a microscope (BX51, Olympus) fitted with a high-speed camera (Miro M310, Phantom). The bubble dimensions and generation frequency were evaluated from the recorded videos using the developed ADM software described in Chapter 4. As the transducer heats up during the operation, a fan (IJMCF-J01BM05-9, Toshiba) was used to provide convection cooling to prevent the temperature at the junction from increasing by more than 2°C in 10 minutes. The temperature was measured by inserting a Type K thermocouple.
into the microfluidic device. The transducer was switched off immediately after each data acquisition to prevent overheating.

5.4. Results

5.4.1. Vibration modes of the transducer system

Monodisperse bubbles are formed under a gas pressure of 45 kPa and an oil flow rate of 960 μL h⁻¹. Subsequently, the size of generated bubbles was investigated under a frequency search from 1–400 kHz with a 1 kHz interval. The bubbles show a significant increase in size when the actuation frequency is tuned to around 128 kHz and 290 kHz. Vibration of gas-liquid interface also occurs around the frequencies. Figure 5-9(a) shows the frequency response of the bubble size in the range of 120 to 136 kHz, which has a sharp peak at 128 kHz; while Figure 5-9(b) is for the range between 280 and 310 kHz, with peak around 290 kHz, lesser in sharpness. Interestingly, as shown in Figure 5-10, the measurement on the electrical impedance on the attached transducer also shows a pair of local minimum and maximum impedance magnitude around 128 kHz and 290 kHz.

![Figure 5-9 Tuning of bubble size over a range of frequency (a) 120–136 kHz (b) 280–310 kHz.](image-url)
Figure 5-10 Electrical impedance of the transducer over a range of frequency (a) 120–136 kHz (b) 280–310 kHz.

Keeping the actuation conditions at 40 V and 128 kHz, the process of the bubble generation was captured by the high-speed camera at a rate of 192,440 frames per second. c4lc01139b2.mp4 [57] shows an oscillatory motion of the gas–liquid interface. The speed of the motion is much faster than the forward movement of the gas–liquid interface. The interface oscillates in the principal volumetric mode as no nodes are observed at the moving interface and the large channel provides the necessary gas source for the volumetric oscillation.

It is believed that 128 kHz is not the resonant frequency of the gas–liquid interface. Firstly, the resonant frequency of the gas–liquid interface depends on its radius of curvature and the fluidic pressure, as indicated in the solution from Rayleigh–Plesset equation [175],

\[
f^2 = \frac{1}{4\rho \pi^2 a^2} \left\{3\kappa \left(p + \frac{2\gamma}{a}\right) - \frac{2\gamma}{a}\right\},
\]

where \(\rho\) is the density of the liquid, \(\gamma\) is the surface tension, \(\kappa\) is the polytropic exponent, \(p\) is the fluidic pressure, and \(a\) is the radius of the interface. The resonant frequency changes as the radius of the gas–liquid interface curvature changes.
continuously throughout the bubble generation process. If the interface is oscillating at a resonant frequency, vigorous motion can only be observed for a short period. However, the video shows that the interface oscillates with the same amplitude throughout the whole process. Furthermore, the frequency would increase when the oil flow rate and gas pressure increase. Yet, experimental results showed that 128 kHz is still the frequency that produces the maximum bubble size over a range of oil flow rate and gas pressure up to 2700 μL h\(^{-1}\) and 90 kPa, respectively, when it is at the resonance of the transducer.

It is hypothesized that the optimal frequency is the mechanical vibration mode of the transducer system. This hypothesis is consistent with the measurement of the electrical impedance of the transducer system, as a pair of electrical resonance and anti-resonance (local minimum and maximum impedance magnitude) is usually found around the mechanical resonance of piezoelectric actuators [176]. As shown in Figure 5-10, the measurement on the attached transducer showed that pairs of electrical resonance exist around 128 kHz and 290 kHz. Therefore, although the effect of acoustic wave resonances along the gas feeding channel cannot be completely ruled out, it is concluded that the vibration modes of the transducer system control the increase in bubble size.

On another note, the electrical impedance changes after attaching the transducer to the attachment. Many of the original electrical modes that exist before the attachment, as shown in Figure 5-11(a), are dampened after attachment of the transducer, as shown in Figure 5-11(b). The electrical modes (around 128 kHz and 290 kHz) left after the attachment coincides and agrees with the result from the tuning of bubble size over a range of frequency.
In a subsequent test, the size of the bubble generated under the same flow conditions was monitored by varying the applied voltage at a fixed frequency of 128 kHz. The insets in Figure 5-12 show the increasing bubble size with increasing applied voltage. The graph in Figure 5-12 shows that the bubble area is a square function of the applied AC voltage. Thus the increase in bubble area is proportional to the energy input through the transducer.

Figure 5-12 Bubble area (cross section along lengthwise direction) versus applied voltage at 128 kHz.
Furthermore, the transducer is positioned so that its center aligns with the center of the cross-junction of the microchannels. When the transducer is not properly centered, the induced streaming reduces significantly which in turn results in a smaller increase in the size of the bubbles produced.

5.4.2. Bubble size response

The role of pressure and flow rate was then evaluated at a fixed actuation condition of 40 V, 128 kHz. For all the tested flowing conditions, the size of the generated bubble was found to increase significantly after the transducer is turned on. Figure 5-13 shows the micrographs of the droplet captured before and after the activation of the transducer, under selected conditions. The measured bubble size for all the tested conditions are plotted in Figure 5-14. In the figure, the data on the blue lines are all measured from the droplet before the activation of the transducer, while the data on the red lines are taken after the activation of the transducer. Within the set of same pressure, the areas increase significantly after the activation. This forms a new line of similar trends on top of the old blue line.

![Figure 5-13 Micrographs of droplet captured before and after the activation of transducer under selected conditions.](image-url)

<table>
<thead>
<tr>
<th>kPa</th>
<th>Off</th>
<th>On</th>
<th>Off</th>
<th>On</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td></td>
<td>460 µL/h</td>
<td></td>
<td>620 µL/h</td>
</tr>
<tr>
<td>45</td>
<td></td>
<td>960 µL/h</td>
<td></td>
<td>1120 µL/h</td>
</tr>
<tr>
<td>65</td>
<td></td>
<td>1600 µL/h</td>
<td></td>
<td>1800 µL/h</td>
</tr>
<tr>
<td>90</td>
<td></td>
<td>2460 µL/h</td>
<td></td>
<td>2580 µL/h</td>
</tr>
</tbody>
</table>
Figure 5-14 Generated bubble size under different flow conditions with and without actuation.

The evaluation is also shown using contour tracing of the expansion process of the gas–liquid interface under various flow conditions, as shown in Figure 5-15. In all the tested conditions, the interface expands upon the activation of the transducer. From the tracing, the contours after activation take a larger footprint (cover area) at the junction than the ones without activation. This shows the expansion of bubble at the junction also contributes to the increment in the bubble size.

Figure 5-15 Contour tracing of the expansion of gas-liquid interface during a cycle of bubble formation, with and without actuation using a transducer.
Figure 5-16 Generated bubble size in dimensionless number under different flow conditions with and without actuation. The insets show the generated bubbles under the selected flow condition.

The results in Figure 5-14 are plotted in Figure 5-16 using dimensionless numbers in which the relationship [177] is shown in equation (5-2) & (5-3),

\[
\alpha \propto \frac{Q_g}{Q_g + Q_l}, \quad (5-2)
\]

\[
\alpha = \frac{A}{hw} \left( \frac{P - P_{\text{min}}}{P_{\text{min}}} \right)^{0.12}, \quad (5-3)
\]

where \(\alpha\), \(Q_g\), and \(Q_l\) are the dimensionless bubble area, the gas volumetric flow rate and the liquid volumetric flow rate, respectively. The variable \(\alpha\) was determined using bubble area \(A\), gas pressure \(P\), channel height \(h\) and channel width \(w\). The second term in the derivation of \(\alpha\) is a small correction factor for pressure, where \(P_{\text{min}}\) is the minimum pressure (20 kPa) used to achieve stable bubble formation and 0.12 is the fitting power for the relationship. Gas flow rate \(Q_g\) was calculated from the generation frequency and the average volume of a bubble. The volume of a bubble is estimated using the following equation mentioned by van Steijnen et al. [173]:

\[
y = 4.0507x + 0.9652
\]

\[
y = 3.6673x + 0.7
\]
\[ V = hA - 2 \left( \frac{h}{2} \right)^2 \left( 1 - \frac{\pi}{4} \right) l \] (5-4)

where \( h \) is the height of channel, \( A \) is the top-view area, and \( l \) is the perimeter of the top-view area. Figure 5-16 shows that the gas flow rate increases when the transducer is turned on. The \( \alpha \) values converge to a line above the line without actuation following a similar gradient.

5.5. Discussion

The increase in the size of the generated bubbles upon the activation of the transducer can be related to the Rayleigh streaming formed around the gas–liquid interface. This streaming is induced by the rapid oscillation of the interface [148] with theoretical streaming velocity [152] shown in the following equation:

\[ \mu_s \sim 2\pi f \varepsilon^2 a , \] (5-5)

where \( \varepsilon a \) is the oscillation amplitude and \( a \) is the radius of curvature. At \( Q_1 = 960 \mu \text{L h}^{-1} \) and \( P = 45 \text{ kPa} \), the theoretical streaming velocity is \( \sim 26 \text{ mm s}^{-1} \) (\( \varepsilon = 0.04, a = 20 \mu \text{m}, f = 128 \text{ kHz} \)). This order of magnitude is comparable to an average oil flow velocity of \( 62 \text{ mm s}^{-1} \).

In order to explain the mechanism, possible path lines of the streaming interaction based on detailed observation of the experimental results is proposed, and shown in Figure 5-17. The path lines are the resulting flows between the convective and oscillating flow. The resulting flow path lines deviate from the convective ones with a net difference in the positive axial direction. This difference promotes a net pumping effect that increases the flow rate of gas and thus increases the generated bubble size.
Figure 5-17 Generic streaming path lines resulting from the combination of convective and oscillating flow.

It is to be highlighted that the streaming path lines formed around the forward moving oscillating gas–liquid interface in a fast flowing medium is a complex problem by itself. The current proposed model is just a reasonable hypothesis which justifies the experimental results. The velocity of the induced streaming around the oscillating interface is in the order comparable to the flow velocity.

Next, a repeatability test was carried out under constant flow conditions ($P = 45$ kPa, $Q_l = 960$ $\mu$L h$^{-1}$). In this test, the transducer was switched on and off every two seconds. Figure 5-18 shows the test results, where 1 and 0 are the on and off state of the transducer, respectively. The size response of the formed bubble to the transducer state is repeatable. The data show a sharp rise or fall in the bubble area around the switching points. The change in bubble size within 100 ms is shown in c4lc01139b3.mp4 [57]. This observation indicates that the induced streaming occurs very quickly after turning on the transducer.
Figure 5-18 Generated bubble size response to the transducer state. Insets show the images captured at off (0) state and on (1) state.

5.6. Summary
A new method to control the generated bubble size in flow-focusing configuration using an ultrasonic transducer has been tested and demonstrated. Acoustic streaming was induced using a forward moving, oscillating gas–liquid interface in the resonance mode of the transducer system. The induced streaming clearly affected the process of bubble formation. The gas flow rate and generated bubble size increased significantly when the transducer is activated. This method was applied and had the same effect on various flow conditions such as gas pressure ranging from 30 to 90 kPa, and flowrate ranging from 380 to 2700 μL h⁻¹. The range of the generated bubble size changes from 3543–10133 μm² without the transducer to 5474–14285 μm² when excited by the transducer. The increase in the generated bubble size was repeatable in seconds. This method is a low-cost and simple way to enhance the capability of a microfluidic bubble generator to produce a wide range of bubble size with feedback control.
Chapter 6: Droplet generation with bubble acoustic control

In the previous chapter, a novel method was tested and demonstrated to control the generation of bubble using an ultrasonic transducer. The setup is able to oscillate a forward moving gas-liquid interface, alter the behavior of the bubble formation and control the size of the bubbles formed. In this chapter, a similar setup is used again to agitate a static gas-liquid interface. A static interface is formed near the droplet formation point. When the interface is agitated by the transducer, streaming around the interface that changes the streamline of the water and can be used to control the formation of droplet.

6.1. Preliminary concept testing

6.1.1. Maintaining static gas-liquid interface

Formation of a static and stable gas-liquid interface is the basic requirement and necessary condition for generating droplets with bubble acoustic control. From the literature, the static gas-liquid interface is usually formed by passing liquids along blind side channels filled with gas [150]. This method is challenging in terms of repeatability and maintainability. Alternatively, the gas-liquid interface can be maintained with a pressure controller. This method permits control of the interface curvature by varying the applied pressure.

6.1.2. Setup

The feasibility of maintaining the interface, using the above method, was tested using a T-junction PDMS device. The device was fabricated using standard soft lithography as described in the appendix. The channel design ($h \approx 28 \mu m; w_{main} \approx 300$
μm; \( w_{\text{side}} \approx 200 \mu m; \) Orifice \( \approx 90 \mu m)\) is shown in Figure 6-1. The fluid was directed into the main channel of the device at a constant flow rate with a syringe pump (neMESYS, Cetoni). The side channel was filled with nitrogen gas at a constant pressure using a pressure controller (PPC4, Fluke). DI water was tested first, followed by mineral oil (M5904, Sigma Aldrich).

Figure 6-1 T-junction channel design for the concept test.

6.1.3. Results
For the test with DI water, a stable interface was maintained at the junction for more than a minute, as shown in Figure 6-2(a). The interface could be balanced either by changing the water flow rate or the gas pressure. It takes more than 3 minutes to form a stable interface when changing the water flow rate. This is because the motion of the interface responds slowly to changes in water flow rate. The response time was much faster when the interface was controlled by changing gas pressure. A balanced interface was achieved in less than a minute using the pressure controller. The flow rate ranges from 100 – 1000 μL/h while the pressure ranges from 5 – 20 kPa.

For another test using mineral oil, either by flow rate or pressure control, the longest duration when a stable interface was maintained was only 9 seconds. This is due to the hydrophobic channel wetting property that promotes the movement of oil-gas
interface. Based on the above outcomes, all subsequent tests were carried out on DI water with gas pressure control.

![Figure](image)

**Figure 6-2** Maintaining stable gas-liquid interface at T-junction (a) water-nitrogen interface (b) oil-nitrogen interface at 9 seconds (c) oil-nitrogen interface after 20 seconds.

### 6.2. Recommended channel designs

The T-junction used in the preliminary concept test cannot evaluate the effect of transducer on droplet generation. Therefore, two different channel configurations were redesigned as shown in Figure 6-3. The two different channel designs were used to evaluate:

(i) Feasibility of maintaining a stable gas-liquid interface

(ii) Feasibility of vibrating the gas-liquid interface with ultrasonic transducer

and,

(iii) Response of droplet generation to vibration

The width and the height of the channel are 100 μm and 45 μm, respectively. Both designs generate water droplets in oil at the cross junction, with different placement of gas-liquid interface. There are two gas-liquid interfaces in design 1, placed symmetrically at both sides of the water channel right before droplet formation junction. Design 2 is similar to design 1, but the number of gas-liquid interface is reduced by one. Furthermore, design 2 has only one input point for oil. The changes in design 2 were made to simplify the flow setup and also to reduce the complexity in maintaining a stable gas-liquid interface. Notice that for both designs, nitrogen
gas was made to pass through a meandering channel before reaching the junction. This was to prevent backflow of liquid into gas tubing in the event of a drastic reduction of the applied gas pressure or increment in the flow rate.

![Diagram of channel designs](image)

**Figure 6-3 Channel designs (a) Design 1 (b) Design 2. Serpentine design on N₂ channel is for easier control of the gas-liquid interface which serves as the buffer when backflow of the interface happens and prevents it from flowing back directly into the input tubing.**

### 6.3. Pressure control

A computer program was written to send remote commands to the controller using an RS232 port. As shown in Figure 6-4, the program integrates remote pressure controller operation function with camera SDK (Miro M310, Phantom). The camera SDK was used to show the live image of the channel and to control the recording function of the camera. In the GUI of the program, there are 16 buttons dedicated to adjust the pressure. There are eight levels of adjustment in either way (i.e. up and down). The levels are 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1 and 2 kPa.
Figure 6-4 GUI of the program integrating remote operation function of pressure controller with camera SDK.

However, even with computer program, it is difficult to maintain both gas-liquid interfaces simultaneously at their junctions using design 1. Usually, one interface lags the other which causes bubbles to form at the junction. Figure 6-5 shows instances where bubbles were formed at one of the junctions. Figure 6-5(a) shows the flowing gas from the bottom side channel passes through the main junction and reaches the outlet channel. Three immiscible fluids: nitrogen gas, mineral oil and water, meet at the main junction. Figure 6-5(b) shows an instance when the gas temporarily blocks water phase from reaching the main junction.

Figure 6-5  (a) Nitrogen gas flows from the bottom side channel through the main junction until the outlet channel. Three immiscible fluids: nitrogen gas, mineral oil and water, meets at the main junction. (b) The gas temporarily blocks water phase from reaching the main junction.

Therefore, design 2 was used in order to reduce the difficulty of maintaining the stable gas-liquid interface at the junction. Also, design 2 requires only one syringe.
pump to drive the mineral oil. The gas-liquid interface was able to balance quickly within a few seconds in design 2. This was made possible only by adjusting the pressure remotely and monitoring/controlling the advancing/receding of the interface.

6.4. Fluid flow setup

![Fluid flow setup](image)

**Figure 6-6 (Left) Fluid flow setup (Right) Micrograph at the junction of the microchannels.**

As shown in Figure 6-6, a water droplet in oil is formed by flowing water to the central channel of the cross junction and mineral oil (M5904, Sigma Aldrich; viscosity $\eta = 30 \text{ mPa s}$) with 5% $\text{w/w}$ non-ionic surfactant (Span 80, Sigma Aldrich) to the two side channels. For both liquids, syringe pumps (neMESYS, Cetoni) were used to provide constant volumetric flow rates. The equilibrium interfacial tension (water-oil) is about 5 mN/m [67]. To keep the gas-liquid interface at one side of the central channel, a pressure controller (PPC4, Fluke) was used to control the pressure of nitrogen gas.

6.5. Results

6.5.1. Vibration of gas-water interface at resonance frequency

The gas-water interface vibrates vigorously at the junction when the ultrasonic transducer is tuned to 128 kHz with power input of 56 $V_{pp}$. Due to the vigorous vibration, the interface can only be maintained at the junction for a few seconds.
before instability sets in. The unstable interfaces (some of them are shown in Figure 6-7) change very quickly from one shape to the other within a few milliseconds.

Figure 6-7 Unstable interfaces.

A more stable interface is achieved by keeping the gas-liquid interface further away from the end of the junction. The interface adjusts itself automatically to a new stable location. The process of the adjustment is captured in the clips shown in Figure 6-8. For a stable interface, the water interface is found to form a bell shape touching the gas interface. There are two gaps in between the gas and the water. It is believed that these are water vapors formed due to the vibration of the interface.

Figure 6-8 Adjustment of interface to become a new stable interface (from top left to top right then bottom left to bottom right).

After the transducer is activated, the interface is found to advance gradually towards the junction. The pressure decreases from time to time to adjust the interface back to the previously equilibrium position. The advancing motion stops after about 10 minutes. The changes in droplet size were then recorded.
6.5.2. Droplet size response

Unlike bubble generation control with ultrasonic transducer in chapter 5, an immediate change in droplet size does not happen after the transducer is activated. The droplet size increases gradually before stabilization in about 10 minutes. Interestingly, the time taken for the advancing gas-liquid interface to stop is also about the same. Figure 6-9 shows the droplet size before and after activation of transducer, under different sets of flow rates.

Generally, droplet size increases slightly after activation of the transducer. On the other hand, the applied pressure decreases significantly, as shown in Figure 6-10. The applied pressure was decreased in order to maintain a stable gas-liquid interface near the junction, as the interface advances gradually after the activation of transducer. Table 6.1 shows the images of the droplet before and after activation of transducer at the selected sets of flow rates, indicated with the applied pressure to maintain a stable liquid-gas interface.

![Figure 6-9 Droplet volume response before and after activation of transducer under different sets of liquid flow rates.](image)
Applied pressure to the nitrogen gas before and after activation of transducer under different sets of liquid flow rates, to maintain the gas-liquid interface at the junction.

Table 6.1 Images of the droplet before and after activation of transducer at the selected sets of flow rates. P is the applied pressure to nitrogen gas to maintain the liquid-gas interface

6.6. The effect of temperature

The vibrating gas-liquid interface has little influence on the droplet size because the size of the droplets took minutes to increase significantly after the transducer is activated and reduced significantly after the transducer is inactive. This phenomenon is very different from the observation discussed in chapter 5 where the response time is in the order of seconds. The buildup heat after activation of transducer and the dissipation of heat after the deactivation is perhaps the most likely factor for the change in droplet size. From the literature [90], the size of droplet generated under the flow focusing channel is subjected to the change in
temperature. It is thus imperative to study the effect of the heat generated by the transducer.

Under the temperature study, a fan (MCF-J01BM05-9, Toshiba) was used to provide convection cooling. The temperature was measured by inserting a Type K thermocouple into the microfluidic device. The gas inlet was blocked for this study in order to eliminate the vibrating interface effect. Droplets were generated with flow rates set of $Q_c=1560 \, \mu\text{L h}^{-1}$, $Q_d=156 \, \mu\text{L h}^{-1}$. Before the measurement, both the fan and transducer were switched on for about 10 minutes at $56 \, V_{pp}$. Afterwards, the fan was on/off at 10 minutes interval. As shown in Figure 6-11, the temperature starts to rise after the fan is switched off. The temperature is brought up by the heat accumulated at the transducer. The droplet volume rises in tandem with the rise in temperature. Right after the fan is switched on, the temperature drops quickly and the droplet volumes also drop in tandem to the decrease in temperature.

![Graph showing droplet volume and temperature over time](image)

**Figure 6-11** Droplet size in response to temperature of the junction.

This temperature study proves that, the change in the droplet size is mainly due to the change in the temperature of the channel, as the result of the heat accumulated
at the transducer. The change in temperature is also related to the change in nitrogen gas pressure required to balance the gas-liquid interface at the junction. This is because both the viscosity and interfacial tension of the mineral oil decreases as temperature increases [84]. When the viscosity drops, the pressure required to drive the same amount of fluids into the same channel drops according to Hagen-Poiseuille equation [178].

6.7. Summary
A method to maintain a stable gas-liquid interface at a junction with acceptable repeatability and maintainability has been developed successfully using a pressure controller with nitrogen gas. By incorporating the gas-liquid interface near the cross junction of the droplet formation point, the effect of the interface vibration by the ultrasonic transducer to the droplet generation process has been studied. Droplet size is found to increase after activation of ultrasonic transducer. The pressure to maintain the gas-liquid interface at its junction also drops after activation of the transducer. Further investigation has shown that, the change in size is directly related to the temperature in the channel, as heats accumulate at the transducer which causes the rise in the channel temperature. It is thus concluded that it is the change in temperature that alters the droplet size.

The temperature study has actually helped to refine the studies for the acoustic control of bubble generation in the previous chapter. It is from this study that the applied voltage to the transducer was reduced from $56 \, V_{pp}$ to $40 \, V_{pp}$ and a fan was used to provide convection cooling. These additional measures help to prevent the temperature at the junction from rising too rapidly.
Chapter 7: Conclusions and future work

7.1. Concluding remarks

In this thesis, major research efforts have been done to achieve the proposed objectives:

(1) To review comprehensively the available active control methods for droplet/bubble generation. Based on the review, a new novel active control method was developed.

(2) To develop a video processing software application that can perform in situ droplet/bubble measurement automatically. The software was expected for:

   a. The evaluation of newly developed active method quickly and efficiently.
   b. Researchers from various different background and disciplines to study droplet/bubble generation.

Both laser engraving and injection molded methods have been explored to fabricate the microchannel in PMMA for droplet generation. Injection molding method was chosen based on the quality of the microchannel produced. The open channel of the PMMA device was enclosed by an adhesive tape. The 3M tape was chosen among different types of tapes after performing a variety of different tests. Multiphasic behavior under the fabricated PMMA device has been studied to find out the characteristic of the flow under different sets of flow rates. The capability of the PMMA device as a droplet generator was evaluated by characterizing the generated droplets under different sets of flow rates. The study has offered the insight of the
limitation and the merits of the fabricated PMMA devices, which is important for deciding which method and material to choose for different purposes.

A new video processing software application (ADM) for *in situ* droplet measurements has been developed. Automated processing step has been achieved with a newly developed object based background extraction operation (BEO) and automated binary threshold value selection (BTVS) operation. ADM also has much higher throughput than any currently available software. As the processing speed is higher than the video transfer speed, it also allows simultaneous processing with video transfer for *in situ* measurement, by integrating with camera software development kit (SDK). The ADM software has been publicly released for free usage and evaluation.

A new active method to control bubble generation using an ultrasonic transducer has been developed. Acoustic streaming is induced using a forward moving, oscillating gas–liquid interface in the resonance mode of the transducer system. The induced streaming affected the process of bubble formation. The gas flow rate and generated bubble size increased significantly when the transducer is turned on. This method was applied and had the same effect to variable flow conditions such as gas pressure ranging from 30 to 90 kPa, and flowrate ranging from 380 to 2700 μL h⁻¹. The range of the generated bubble size changes from 3543–10133 μm² without the transducer to 5474–14285 μm² when excited by the transducer. The increase in the generated bubble size is also repeatable in seconds. This method is a low-cost and simple way to enhance the capability of a microfluidic bubble generator to produce a wide range of bubble size with feedback control.

A method to maintain gas-liquid interface at a junction with acceptable repeatability and maintainability has been developed successfully using a pressure controller
coupled to the nitrogen gas. By incorporating the gas-liquid interface near the cross junction of the droplet formation point, the effect of the interface vibration by the ultrasonic transducer to the droplet generation process has been studied. Droplet size is found to increase after activation of ultrasonic transducer. The pressure to maintain the gas-liquid interface at its junction also drops after activation of the transducer. Further investigation shows that the change in size is related to the temperature in the channel. The change in temperature is due to heat accumulation at the transducer interface.

7.2. Contributions

The key contributions of the thesis are summarized as follow:


2. A new video processing software (ADM) that is able to measure droplet parameters automatically. The processing speed is also significantly faster than any available software. The software is freely available at http://a-d-m.weebly.com.

3. Fabrication of a PMMA microfluidic droplet generator and evaluation of the capability of the device which offered the insight of the limitations and merits of the PMMA devices.

7.3. Recommendations for future work

From the study on the capability of the PMMA device as a microfluidic generator, adhesive tape was found to impede the range of the applicable flow rates for monodisperse droplet generation. The main limiting factor is adherence of the generated droplet to the adhesive material on the tape. The capability of the PMMA
device can be improved further by finding a suitable material to coat the bonded channel walls or developing other bonding methods that do not distort the channel.

In the thesis, integration of camera SDK with ADM software was hard-coded into the ADM. It currently supports only specific brand of cameras. To overcome this limitation, ADM needs to be further modularized so as to allow interfacing with external plugins for video input from camera. The external plugin can then be developed by interested parties to expand ADM’s support for other cameras. Besides, modularization of ADM could also be made to allow plugins for customized filters. The customized filter will expand the capability of ADM to recognize the droplets in specific situations such as touching droplets.

During the development for active control of bubble generation using an ultrasonic transducer, the generated bubble was found to switch from one shape to the other when the ultrasonic transducer is turned on and off periodically. For a significant effect, the input voltage to the transducer has been increased to be higher than the voltage recommended by the manufacturer. More study can be done to characterize and explain the phenomena using a higher power ultrasonic transducer. Besides, a detailed simulation can also be done to understand the induced streaming path lines induced by the transducer at the junction which causes the change in generated bubble size.

For droplet generation with bubble acoustic control, a higher power ultrasonic transducer can be used to increase the effect of vibrating gas-liquid interface to change in droplet generation process. Creation of more junctions for the static-liquid interface can also help to amplify the effect of bubble acoustic. The design of the junctions can be improved for more stable formation of gas-liquid interface. The
maintenance of the interface can be done automatically using feedback control to study bubble acoustic control more effectively.
Engraving PMMA sheet using CO$_2$ laser

A monochromatic light emitted by a CO$_2$ laser beam can be focused down to a small spot with high energy density. When the spot is directed onto a thermoplastic material, it induces a localized melt and thus creates channels on the surface. As the spot size of the commercially available CO$_2$ laser systems are usually smaller than 0.2 mm, there is a potential of using the systems to fabricate microchannel with cross section of lesser than 400 x 400 μm. Therefore, the use of a CO$_2$ laser system was studied to explore the possibility of fabricating a microfluidic droplet generator. Epilog Legend 32EX was used in the study. Table 7.1 shows its specification of an Epilog Legend 32EX machine.

<table>
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<tr>
<th>Maximum engraving area</th>
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<td>Operating modes</td>
<td>Raster, vector or combined raster/vector mode</td>
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<td>Depth of field</td>
<td>± 2.54 mm</td>
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Table 7.1 Specifications of Epilog Legend 32EX

The laser system was used to engrave microchannel on a surface of a PMMA sheet with thickness of 1mm (Acrylic Sheet, Ying Kwang Acrylic). For this study, vector mode was used instead of raster for better resolution. Under this mode, the dimension and the quality can be changed by varying the engraving speed and the power of the laser. In order to produce channel with small width, only the lowest laser power (2.25 W) and its immediate higher power (3 W) were used. The engraving speed was set to vary from 240 to 1280 mm/s. Table 7.2 shows the final
Table 7.2 Result of laser engraving on a PMMA sheet under different powers and engraving speeds

<table>
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<th>Speed (mm/s)</th>
<th>Power=2.25 W</th>
<th>Power=3 W</th>
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<tr>
<td>1280</td>
<td>(k)</td>
<td>192 (l)</td>
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</table>

results. Generally, within the same engraving speed, the width of channel increases as the power is increased. This is because more areas are melted as the laser power
is increased. On the other hand, within the same power, the width decreases as the engraving speed is increased. This can be related to lesser laser energy received by the material, as the exposure time is shortened.

The system starts to produce wavy channel at higher engraving speed, from 480 and 640 mm/s at 2.25 and 3 watt respectively. Further increment in the speed produces wavy channels [Figure (c) & (f) in Table 7.2] with increased wavelength. At 2.25 watt, dotted engraves [Figure (i) & (k) in Table 7.2] are produced at the speed of 960 and 1280 mm/s. The production of wavy and dotted engraves can be attributed to the nature of pulse width modulation (PWM) used to control the power of laser system. This occurs when the engraving speed is relatively higher than the frequency of PWM set in the system. The variation of laser power experienced by the surface across a straight line is especially large when the engraving is done at higher speed under low power.

All the engraved surfaces are rough. Figure 7-1 shows the profile of a channel done with 2.25 watt and 800 mm/s using a confocal imaging profiler (PLμ, Sensofar).

![Figure 7-1 Profile of engraving (speed=800 mm/s, power=2.25 W) surveyed using a confocal imaging profiler.](image-url)
This brief study has demonstrated that it is impractical to achieve the optimum combination of speed and power to engrave microchannel on the surface of PMMA. The setbacks of the system are: (i) unable to produce a straight channel which is less than 400 µm in width; (ii) rough surface finish and (iii) V-shape cross-section. These can be contributed by several factors. The first factor is the use of relatively large laser spot size (127 µm) to produce channel with width less than 400 µm. Next, the intensity profile of the CO₂ laser beam is focused at the center. Furthermore, the intensity profile of the laser spot is non-uniform within the same radius [179], as shown in Figure 7-2.

![Figure 7-2 Intensity profile of a laser spot.](image)

In addition, even with a laser machine that has higher PWM frequency (Universal M300, MarkIDent) than the one tested, the surface roughness obtained from the literature [180] is still not desired for droplet generator. Rough channel surface affects the generation of monodispersed droplets as the produced droplets could be broken up while traveling downstream. It is also hard to maintain consistence travelling speed along the channel. Furthermore, measurement of droplet size cannot be done without fluorescent dye due to the poor surface quality [181]. Although effort has been made to decrease the wall roughness by using unfocused laser beam [182], the results is still not suitable as the depth and width of the ablated channel are hard to control independently.
Fabrication of PDMS device

Fabrication of PDMS device starts by making a mold using the SU-8 photoresist. The microstructured mold is then replicated by pouring and curing liquid PDMS mixed with a cross-linking agent. At the end of the process, an elastomeric replica is produced. The detailed of the process will be discussed in the following subsection.

Mold making with SU-8 photoresist

After cleaning and drying a silicon wafer (4 inch, <100> type), SU-8 photoresist is coated on the polished side of the wafer by spin coating. Then, the wafer is soft baked to evaporate the solvents in the photoresist. After cooling down the wafer, the photoresist is exposed under ultraviolet light (365 nm) through a plastic photomask to pattern the microfluidic channels. This is continued by post exposure baking to complete the polymerization. The photoresist on the wafer is then developed upon cooling down in order to wash away the unexposed parts, leaving only the patterned microchannels structure. Hard baking is then followed to further cross link the left photoresist structure. Apart from improving the mechanical properties of the structure, hard baking can also decrease the surface roughness of the channels.

The quality of the structure is sensitive to the conditions of the process. Figure 7-3(a) shows an unsatisfactory structure quality, produced before optimizing the parameters of the mold making process. The figure shows the cross junction of droplet generator pattern, with width of 100 µm, height of 45 µm. The edges of the structure are rough. Although the edges can be smoothed up by hard baking, the top part becomes rounded, as shown in Figure 7-3(b). This affects the precision of the fabrication. Figure 7-3(c) shows a much better structure produced after optimizing the parameters of the process.
Figure 7-3 Patterned SU-8 with microchannel for droplet generation on a silicon wafer. The micrographs are taken at the cross junction.

**PDMS casting**

The mold is placed on a petri dish with the patterned SU-8 structure faced up. Afterwards, PDMS mixed with curing agent at weight ratio of 10:1 are poured on top of the mold. The PDMS mixture has been degassed with vacuum desiccator prior to pouring. The weight ratio for the mixture can be reduced to 5:1 to make a more rigid device. After removing the remaining bubbles from the mixture, the petri dish is placed in an oven to cure for 2 hours at 80°C. The cross-linked PDMS is then trimmed to the desired size. The inlets and outlets of the channels are then made by punching on the trimmed device.
Air plasma bonding for PDMS device

By treating PDMS device and glass slide with air plasma, a covalent bond between two different materials is achievable when the treated surfaces are joined. The pressure used to create the air plasma in the plasma cleaner (PDC-32G, Harrick) is around 65 Pa. The bonded device is then baked at 80°C in an oven for 6 hours to restore the hydrophobicity of the PDMS device. The heat treatment in the final stage is to ensure the bonded devices have consistent surface property.
Droplet generation in PMM device

Continuous phase: Mineral Oil with 1 wt% Span 80, Dispersed phase: DI water

Source of mineral oil: Light Mineral Oil (330779, Sigma-Aldrich, Singapore)
Source of Span 80: Span 80 (Sigma S6760, Sigma-Aldrich, Singapore)

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- **Threading**
- **Varicose**
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- **Stratification**
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References


