19th Australasian Fluid Mechanics Conference
Melbourne, Australia
8-11 December 2014

On-Chip Biochemistry: Droplet Generation and Control

A. Neild[1], D. J. Collins[1], M. Sesen[1] and T. Alan[1]

[1]Laboratory for Micro Systems
Department of Mechanical & Aerospace Engineering
Monash University, Clayton, 3800, Vic, Australia

Abstract

Digital microfluidics, the use of small sample droplets in oil filled channels, offers exciting possibilities in biochemistry. With the ability to form nano or picolitre droplets, there is potential for massive miniaturisation of high throughput screening (HTS) processes, where the full functionality of microtitre plate technology is replicated on a single chip for improved efficiency and substantially reduced costs. To implement such a system, the ability to create reagent mixtures on-chip in multiple permutations is required. For this goal, conventional droplet formation approaches are not well suited, where streams of hundereds of single-reagent droplets are created continuously, whereas applications such as HTS require the time-controlled creation of individual droplets. In this work, we examine the use of surface acoustic wave (SAW) excitation as a method to first generate single droplets on-demand, and then control their subsequent behaviour such that a platform for on-chip HTS can be developed. In addition, we examine methods of particle handling suitable for their introduction, in small numbers, into droplets, with the future potential for single cell encapsulation.

Introduction

Digital microfluidics encompasses small scale fluid systems which use two immiscible phases. The key concept being that small sample droplets can be held isolated from each other by an immiscible buffer, usually oil based. The field has received significant attention because of the promise of on-chip biochemistry, where each droplet can be essentially be considered as a very small test-tube, albeit a far smaller (on the scale of nanoliters) and therefore cheaper one.

The starting point for digital microfluidics is the generation of accurate, uniform and monodisperse droplets. Existing approaches use continuous hydrodynamic techniques; a range of geometries have been developed including 1) junctions[1] at which the two immiscible fluids flowing through perpendicular micro-channels meet to generate droplets, 2) flow focusing devices[2], where the dispersed phase is pinched off orthogonally by two continuous phase streams that are flowing in towards each other, and finally 3) co-flowing streams[3] which exploit the instabilities arising between the two continuous flowing phases. The common feature among these techniques, however, is that a continuous stream of droplets is formed.

While there are a multitude of applications for such systems, it is highly problematic to gain the flexibility required to react one library of chemicals with another (as in HTS) when thousands of identical droplets are generated. To this end, we present a method to produce one droplet at a time, using surface acoustic wave (SAW) excitation. In addition, we demonstrate that merging of two droplets can also be achieved on-demand using this form of excitation.

Acoustic Actuated Microfluidics

The use of ultrasound in microfluidic systems has become well established and has already produced many technologies including particle concentration, particle sorting, mixing and atomization. SAW-based systems are a subset of this field, defined by the manner in which the ultrasound is generated. An AC signal is applied to interdigital transducers (IDTs) – periodic electrodes patterned on a piezoelectric substrate – where the frequency of excitation is dictated by the pitch between the IDTs.

In our systems, the surface acoustic waves couple into the fluidic channels which are bonded onto the piezoelectric substrate; this causes an ultrasonic sound field in the fluid. The sound field, in turn, generates acoustic radiation forces (ARF). The origin of ARF is unintuitive and will be explained in the more common scenario of particle manipulation. For a particle to migrate in an ultrasonic field a second order forcing mechanism is required, this comes in the form of ARF. When an ultrasonic wave propagating in a fluid impinges on a solid particle, the acoustic impedance change at the fluid/solid interface will cause scattering and so a pressure gradient over the surface of the particle. Considering the forces acting on the surface of the particle, first order effects (i.e. effects taking place at the same frequency as the cycle) such as the differences in pressure around the surface of the particle will reverse every half cycle. However, second order effects cause steady state forces, in which there is a net non-zero time-averaged component over a cycle. Such second order terms arise in the Navier-Stokes equation. In this work, the sound field gradient occurs across the oil/water interfaces, and as such the acoustic radiation forces act to deform the interface in the case of droplet generation, and trap the droplet in the case of droplet merging.

Droplet Generation

The system developed[4] uses 40/80 μm wavelength focused SAW devices, comprising 90/45 finger-pairs. These finger pairs were curved forming a 90˚ circular arc, resulting in a focussed SAW at the oil/water interface, as seen in Figure 1. These focussed interdigital transducers (FIDTs) were formed on a 0.5 mm thick, single side polished 128˚ Y-cut, X-propagating lithium niobate (LN) substrate. The 10 nm chrome/200 nm aluminium FIDTs were aligned on the substrate symmetrically with respect to the preferred propagation direction on the LN. With the exception of the electrode pads, the devices were further coated with 70 nm of evaporation-deposited SiO2 to promote adhesion with polydimethylsiloxane (PDMS), which was bonded after exposure to an activated air plasma (450 mTorr, 29.6W, 3 minutes for the SiO2-coated LN substrate and 1.5 minutes for the PDMS). The

PDMS was cast on an etched silicon substrate, allowing definition of the channel geometries.

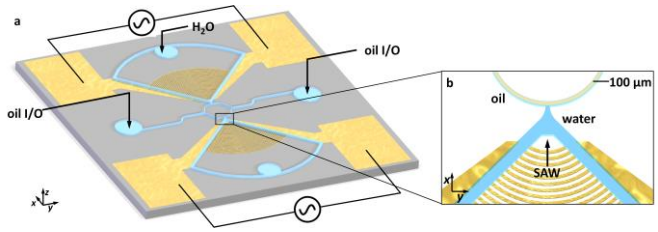

Figure 1: (a) A schematic showing the key features of the SAW droplet generation system. A continuous flow of oil passes through the central channel. (b) Water droplets can be injected into this oil stream by application of SAW focussed at the interface[4].

The small chip was held on the stage of the microscope (Olympus BX43, Tokyo, Japan) using a 3D printed platform. Images were acquired using a 5MP eyepiece camera (AM7023B, Dino-Lite, New Taipei City, Taiwan). The continuous oil flow was induced by using an off-chip syringe pump (KD Scientific 210, Holliston, MA, USA) whereas water (Milli-Q 18.2 MΩ.cm, Millipore, Billerica, MA) was manually manipulated using a 1 mL syringe until a steady-state oil-water interface was achieved. To generate the SAW, an electrical signal was applied from a signal generator/amplifier system (BelektroniG F10, Freital, Germany).

When the SAW is applied to the interface between the water (disperse phase) and flowing oil (continuous phase), the interface deforms. This deformation can be sufficient to cause the water to momentarily block the oil filled channel, at which point a water droplet is pinched off by the pressure gradient induced by the flowing oil. This process can be observed in the images shown in Figure 2. The orifice defining the interface has a width of 20 µm, whilst the channel width and height is 30 µm. The SAW wavelength, defined by the pitch of the IDTs is 80 µm which corresponds to an excitation frequency of 48.4 MHz.

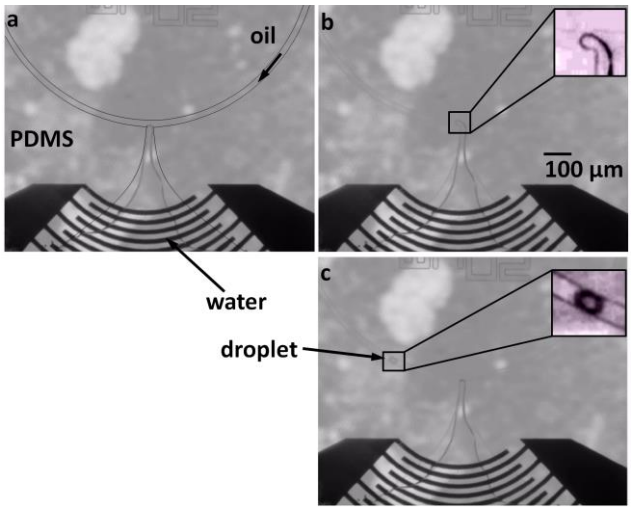

Figure 2: Images of the production of an individual droplet. (a) Prior to application of the SAW a stable interface exists between the water and oil at the opening between the water filled chamber and channel containing the flowing oil. (b) Upon application of the SAW the interface deforms, squeezing out a volume of water into the oil filled channel. (c) subsequently this pinches off from the main water volume, and a droplet is carried down the channel by the oil flow[4].

The droplet sizes that can be produced has been characterised, the key parameters being the duration and power of the SAW pulse. The trends, as shown in Figure 3, show that for higher power and duration larger droplets are produced. The upper limit to this trend occurs when the pinching off process causes the production of two or three droplets. A lower limit occurs when the deformation of the interface isn't sufficient to momentarily block the channel; under this scenario no droplet is produced. It can be seen that range of droplet sizes produced is from 10-30 picoliters for SAW pulse durations from 50-600 ms.

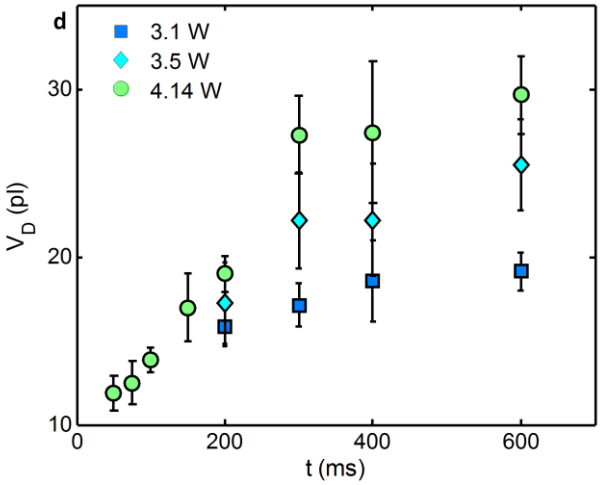

Figure 3: The range of droplet sizes and relationship between size, power and pulse duration is shown. A minimum droplet size of 12 picolitres was produced[4].

An additional capability of this system is in the encapsulation of particles, where the same acoustic radiation force that acts on the water/oil interface can also be used to cause the migration of particles. The same arrangement can therefore cause particles, suspended in the water sample, to migrate to the oil/water interface at relatively low powers. With subsequent application of a power suitable for droplet production, these migrated particles can then be encapsulated in a small droplet[4].

Droplet Merging

In order to enable the reaction of one library of chemicals with another, we have demonstrated the ability to produce single droplets, hence control can be obtained in the sequencing of droplets passing along a channel (not all droplets need be the same as occurs in continuous production methods). However, it is also necessary to be able to merge selected droplets in order to initiate the reactions; this capability is now examined, again by use of SAW actuation so that integration of the capabilities into a single chip becomes straightforward.

In the device presented[5] focused IDTs (FIDTs) of 80 µm wavelength deposited on LN are also used. The PDMS defined channel consists of enlargement fluid expansion zone at the location of the focal point of the IDTS. It is in this zone that droplets can be held against the flowing oil stream. This expansion results in lower local flow velocity for a given volumetric flow rate, yielding a slower moving droplet as well. The application of acoustic radiation forces by excitation of SAW then provide enough force to retain the droplet from this slowed state. When a subsequent droplet arrives, it will merge with the held droplet; due to the volume increase the drag of the oil will then carry the combined droplet downstream. This process is show in Figure 4.

The power required to capture droplets of different sizes has also been investigated, as shown in Figure 5. It has been shown that, by using sufficiently small initial droplets, the number of droplets that can be merged into the same volume is also tunable, with the merger of up to four droplets demonstrated here.

The drag force on a stationary sphere confined in a circular pipe was formulated analytically by Haberman and Sayre[6]. This formula was used as a fit for the transitions in the experimental data shown in Figure 5, where the conditions giving rise to different numbers of merging droplets are shown. Here, the drag force is equated to the acoustic radiation force, this being proportional to the power applied. Though it should be noted that Haberman and Sayre's assumptions do not match perfectly with the proposed system, it is nevertheless useful here to understand the obtained results, especially with regard to the non-linear increase in the drag force.

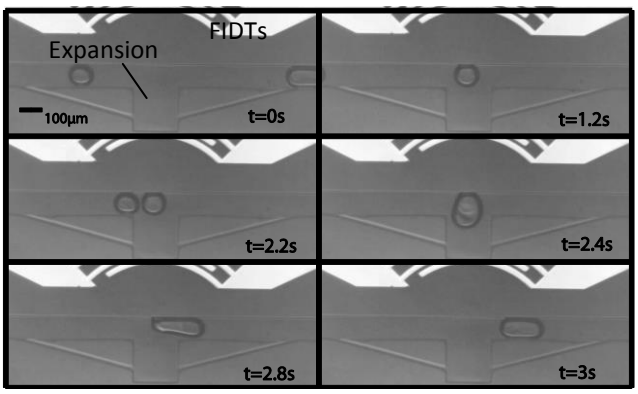

Figure 4: Droplet merging is shown using a time series of images. The first droplet is held in the expansion chamber, t = 1.2s, which is opposite the focussed IDTS (the last fingers of which are seen at the upper part of the images). When the next droplet arrives, t = 2.2s, they merge, t = 2.4s, at which point the drag caused by the oil flowing around the large volume in such a confined space causes the droplet to flow downstream, t = 2.8s[5].

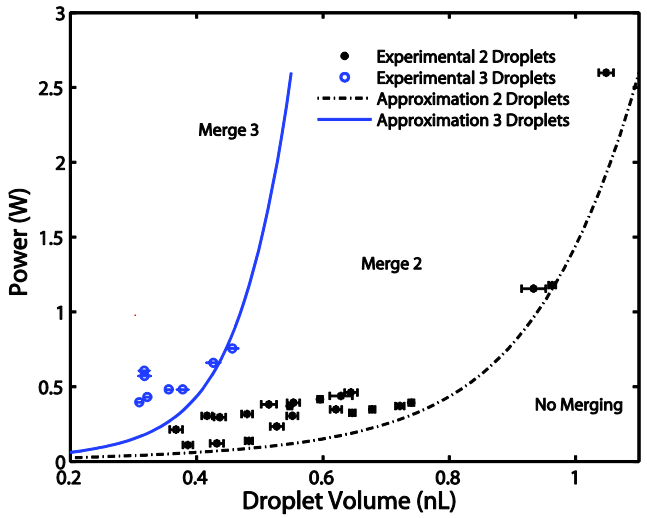

Figure 5: Data points for the minimum required actuation power to trap droplets are shown. Black data points (asterisks) represent the merging of two droplets of the shown volume, whereas the blue data points (circle) are for merging three consecutive droplets of the shown volume. Droplet volumes are calculated via video analysis for a minimum of three times, standard deviations shown. Curve fits based on Haberman and Sayre's[6] analytical drag coefficient for a sphere confined in a circular tube are also plotted as approximating curves for merging of two (dash-dot) and three (solid line) consecutive droplets, thus, regions where merging of none, two or three droplets will occur are predicted.

Consider the critical volume/power ratio, at which the minimum ARF condition is met to retain a droplet in the focal point of the acoustic beam. When the next incoming droplet arrives this critical ratio is exceeded, resulting in the release of a merged droplet with a volume equal to that of the contributing droplets. If three droplets are to be merged, then the initial droplet volume must be less than half of this critical volume, such that the first merging event renders a volume which can still be held by the acoustic forces, prior to the second merging event causing the departure of the three now-merged droplets. This consideration was used to fix the location of the blue transition line (two to three droplets) with respect to the block dotted line (maximum volume for a single merging event).

CONCLUSION

Focussed SAW has been demonstrated here for the on-demand production of individual droplets, where the droplet volume can be parametrically controlled by the duration and power of acoustic excitation down to as low as 12 picoliters. Using a similar excitation arrangement, we have also demonstrated that droplets can be merged together by use of an acoustic trap, again excited by pulses of SAW. Here, the volume of the trapped droplet and the number of droplets to be merged prior to release dictates the minimum actuation power required. Combined, these techniques represent a promising new paradigm for controlled biochemistry reactions on-chip, vital for the testing of chemical libraries for HTS.

Acknowledgments

The devices developed for this work have been fabricated at the Melbourne Centre for Nanofabrication.

References

[1] Thorsen, T., Roberts, F. H., Arnold, F. H. & Quake, S.R., Dynamic pattern formation in a vesicle-generating microfluidic device, *Physical Review Letters*, **86**, 2001, 4163-4166.

[2] Dreyfus, R., Tabeling, P. & Willaime, H., Ordered and Disordered Patterns in Two-Phase Flows in Microchannels, *Physical Review Letters*, **90**, 2003, 144505.

[3] Cramer, C., Fischer, P. & Windhab, E. J., Drop formation in a co-flowing ambient fluid, *Chemical Engineering Science*, **59**, 2004, 3045-3058.

[4] Collins, D., Alan, T., Helmerson, K. & Neild,A., Surface acoustic waves for on-demand production of picoliter droplets and particle encapsulation, *Lab on a Chip*, **13**, 2013, 3225 - 3231.

[5] Sesen, M., Alan, T. & Neild, A., Microfluidic on-demand droplet merging using surface acoustic waves, *Lab on a Chip*, **14**, 2014, 3325-3333.

[6] Haberman, W. L. & Sayre, R. M., *Motion of Rigid and Fluid Spheres in Stationary and Moving Liquids*, David Taylor Model Basin Reports, 1958.