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Daniel Ahmed

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The dissertation of Daniel Ahmed was reviewed and approved* by the following:

Tony Jun Huang
Professor of Engineering Science and Mechanics
Dissertation Advisor
Chair of Committee

Bernhard R. Tittmann
Schell Professor of Engineering Science and Mechanics

Corina Drapaca
Professor of Engineering Science and Mechanics

Peter J Butler
Associate Professor of Biomedical Engineering

Judith A. Todd
Professor
P. B. Breneman Head of the Department of Engineering Science and Mechanics

*Signatures are on file in the Graduate School
ABSTRACT

The physics governing swimming at the microscale—where viscous forces dominate over inertial—is distinctly different than that at the macroscale. Devices capable of finely controlled swimming at the microscale could enable bold ideas such as targeted drug delivery, non-invasive microsurgery, and precise materials assembly. Progress has already been made towards such artificial microswimmers using several means of actuation: chemical reactions and applied magnetic, electric or acoustic fields. However, the prevailing goal of selective actuation of a single microswimmer from within a group, the first step towards collaborative, guided action by a group of swimmers, has so far not been achieved. Here I present a new class of microswimmer that accomplishes for the first time selective actuation (Chapter 1). The swimmer design eschews the commonly-held design paradigm that microswimmers must use non-reciprocal motion to achieve propulsion; instead, the swimmer is propelled by oscillatory motion of an air bubble trapped within the swimmer’s polymer body. This oscillatory motion is driven by a low-power biocompatible acoustic field to the ambient liquid, with meaningful swimmer propulsion occurring only at resonance frequencies of the bubble. This acoustically-powered microswimmer performs controllable rapid translational and rotational motion even in highly viscous liquid. By using a group of swimmers each with a different bubble size (and thus different resonance frequencies) selective actuation of a single swimmer from among the group can be readily achieved.

Cellular response to chemical microenvironments depends on the spatiotemporal characteristics of the stimulus, which is central to many biological processes including gene expression, cell migration, differentiation, apoptosis, and intercellular signaling. To date, studies have been limited to digital (or step) chemical stimulation with little control over the temporal counterparts. Microfluidic approaches have offered a higher level of sophistication in terms of
liquid manipulation, however, due to low Reynolds number associated with these methods, precise temporal manipulation has remained a challenge. Furthermore, varying the sample concentration rapidly and controllably, an important task for a plethora of chemical and biological studies, has proven to be extremely difficult. Here I demonstrate (Chapter 3) a novel approach for generating chemical waveforms that permits continuous modulation of the signal characteristics including the shape, frequency, amplitude (sample concentration), and duty cycle, with frequencies reaching up to 30 Hz. Furthermore, using multiple bubbles of different sizes in a single microchannel, we show fast switching between multiple distinct stimuli, wherein the waveform of each stimulus is independently controlled. Using our device, we characterized the frequency-dependent activation and internalization of the $\beta_2$-adrenergic receptor ($\beta_2$-AR), a prototypic G-protein coupled receptors (GPCRs) due to epinephrine. We determined that $\beta_2$-AR internalization due to epinephrine occurs on timescales between 100 ms and 5 sec. The chemical waveform generation and switching method presented herein is expected to be useful for understanding the dynamics of fast biomolecular processes.
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DEDICATION

I dedicate my work to my beloved uncle Saiful Kader and my most loved younger brother Siam (Adnan Ahmed).
Chapter 1

Selective manipulable acoustic powered microswimmer

1.1 Introduction

The physics governing swimming at the microscale—where viscous forces dominate over inertial—is distinctly different than that at the macroscale. But if we can master these physics and create devices capable of finely controlled swimming at the microscale, we can enable bold ideas such as targeted drug delivery [1], non-invasive microsurgery, and highly precise materials assembly. Some progress has already been made towards such artificial microswimmers, using various means of actuation: chemical reaction [2–5], applied magnetic, acoustic and electric fields [6–12]. However, the prevailing goal of selective actuation of a single microswimmer from within a group—the first step towards collaborative action by a group of swimmers—has so far not been achieved. In this chapter, we present a new class of microswimmer, accomplishing for the first time selective actuation. Our swimmer design eschews the commonly-held design paradigm that microswimmers must use non-reciprocal motion to achieve propulsion; instead, the swimmer is propelled by oscillatory motion of an air bubble trapped within the swimmer’s polymer body. This oscillatory motion is driven by application of a low-power acoustic field (i.e. not damaging to cells) to the ambient liquid, with meaningful swimmer propulsion occurring only at resonant frequencies of the bubble. This acoustically-powered microswimmer accomplishes controllable and rapid translational
and rotational motion, even in highly viscous liquid. And by using a group of swimmers each with a different bubble size—leading to different resonant frequencies—selective actuation of a single swimmer from among the group can be readily achieved.

1.2 Motivation

Microrobots or microswimmers can potentially be utilized in numerous applications ranging from small particles and cells manipulation, oil recovery, sensor applications and numerous in vitro chemical and biological experiments. Robust, simple operation, untethered and biocompatible microswimmer can revolutionize the concept of human surgery. Traditional surgery requires large cut for the surgeon to operate, where the damage is done in the skin, muscle, connective tissue and bone to reach the targeted region and often causing greater injury than the curative procedures [13]. Thus contributes more pain and elongated recovery times to patients [13]. On the other, non-invasive method will allow faster recovery time, less medical complications and infection risk and reduced trauma [13–15]. In addition, from the surgeons point of view, minimally invasive procedures may reduce the dexterity required in a regular operation thus reducing the errors rising from fatigue or stress [13]. Recently, Nelson et al. laid out some potential medical tasks which can be carried out in-vivo in the near future, they include specific drug delivery to a tumor cite, brachytherapy, stents and stem cells drafting, biopsy, ablations, etc., a whole list is shown in Figure 1-1 [14].

Though millimeter to centimeter sized capsule has been utilized to track images of the digestive track, large scale swimmers are usually disadvantageous to access different
parts of a human body, such as the central nervous system, urinary track or to navigate through different vessels. Batteries, the driving source of a millimeter sized swimmers, have proven to be miniaturized [14] thus limiting the size of a swimmer. An alternative way to maneuver a swimmer is to harness the energy from the environment or from the external driving fields.

Figure 1-1 Potential noninvasive medical applications that can be utilized by the advent of microswimmers. (a) Targeted drug delivery. (b) Brachytherapy to kill targeted tumor cells. (c) Hyperthermia to kill wanted cells. (d) Stem cell in vivo drafting. (e) Ablation. (f) Biopsy. (g) Scaffolding. (h) Stents implant. (i) Occlusion. (j) Electrodes implantation in brains. (k) Sensing. (l) Marking [14].
1.3 Background literature

In his classic paper "Life at low Reynolds number", Purcell proposed the "scallop theorem," which states that the reciprocal motion of a two-state motor submerged in liquid at low Reynolds number results in no net motion through the liquid [6], [16]. This scallop theorem is demonstrated by nature’s microswimmers example includes *Escherichia coli*, *Spirillum Volutans*, spirochetes and sperm cell, as shown in Figure 1-2, which achieve propulsion through the liquid environments by means of flagella, cilia, and other appendages that produce non-reciprocal motion. To date, researchers have used Purcell’s scallop theorem—and the natural microswimmers that prompted Purcell to formulate it—as a design paradigm for propulsion at the micro scale: the resulting artificial microswimmers have all sought to achieve non-reciprocal motion.

![Figure 1-2 Non-reciprocal motion of Escherichia coli, Chromatium okenii and Spirillum volutans [16].](image-url)
1.3.1 Chemical based swimmers

As of yet there exist numerous artificial based swimmers, which are propelled in the presence of external fields (electric, magnetic or acoustic) or by inducing chemical reaction. Chemical based swimmers are attractive, since they don’t require any moving parts. Wilson et al. demonstrated a novel swimmer, which are called polymer stomatocytes [2]. Stomatocytes are bowl-shaped nanoscale structures fabricated by deformation of the polymer vesicle [2]. The swimmer consists of a nanocavity containing catalytically active platinum nanoparticles, which is then immersed in hydrogen peroxide solution. As a result, the catalyst (platinum nanoparticles) decomposed hydrogen peroxide into oxygen and water as shown in Figure 1-3 (a). The oxygen molecules in the form of bubbles are rapidly discharged from the narrow opening of the swimmers, thus producing a thrust. The motion of the swimmer was directed since it propel in the direction opposite to the jetting direction. Subsequent motion of stomatocytes were tracked and represented in Figure 1-3 (b). Recently, Ikezoe et al. demonstrated a novel autonomous biochemical motor by introducing metal-organic framework (MOF) structure to achieve efficient propulsion [4]. The MOF consists of nanosized mesh, which they have utilized as a dense storage capacity of peptides molecules, Figure 1-4 (a). When the swimmer containing MOF filled with peptides molecules was placed at the air/water interface, the molecules were released in a directional orientation into the water, as shown in Figure 1-4 (b). The release of the peptides molecules creates a surface tension gradient, causing it to propel from low to high surface tension gradient. The swimming velocity of the swimmer was characterized in Figure 1-4 (c). Due to the
integration of MOF structures, the swimmer was observed to propel efficiently for 10 minutes. However, the directionality of the motion of the swimmer is compromised by the initial impulse of the peptide molecules emission [4].

Figure 1-3 (a) Schematic shows the propulsion mechanism of nanosize stomatocytes. (b) Micrograph shows the tracked motion of multiple stomatocytes visualized by different colors [2].

Figure 1-4 (a) Swimmer containing MOF filled with peptide molecules. (b) Release of peptides molecules from the swimmer at the water interface. (c) Swimmer’s velocity characterization [4].
Harnessing energy from the environment to propel a swimmer is advantageous; however precise control in terms of directionality has so far remained a challenge. To address some of the issue and the challenges, I have developed a novel chemical based swimmer by introducing microchannels. The swimmers were fabricated by photopolymerization method, where the swimmers take the shape of the mask design [17]. A flat triangular swimmer starts propelling due to surface tension gradient when displaced at the water/air interface in a petri dish, Figure 1-5 (a)-(b). To our surprise, we observed dual nature of this swimmer. The swimmer propels continuously with a velocity that decays exponentially for the first ~ 50 seconds, after which it undergoes well-defined pulse motion. The peak-to-peak velocity of the pulse motion remains constant at 12 cm/s; however the duration of each pulse increases over time, as demonstrated in Figure 1-5 (e). The dual nature of motion was observed for different shaped swimmers such as flat circular, rectangle and tear drop; however their motion was observed to be random. To minimize the randomness, I have introduced a pair of serpentine channels (of 10 µm in width) on the flat triangular body, consequently the swimmer propel with a dedicated head and a tail. The surface active materials were ejected out from the outlet of the serpentine channels and since the channels span out the whole top surface area of the swimmer, it propels continuously along it entire path, as shown in Figure 1-5 (c) and (e). However, due to high Reynolds number associated with the swimmer, stability has remained a challenge. To address the issue, I have designed and fabricated a bird-shaped swimmer with parallel microchannels on the wings and on the tail. Consequently, the swimmer eject the surface active material from the channels, thus allowing it to propel linearly as shown in Figure 1-5 (d) and (e).
Figure 1-5 Propulsion of chemical based surface swimmers. (a) Micrograph shows a stationary triangular swimmer at the water/air interface in a petri-dish filled with water. (b) Dual propulsion behavior of a flat triangular swimmer. The trajectory of the swimmer was marked by initial continuous but random motion (red), which later shown to propel in a distinct pulse motion (yellow) demonstrated by “0, no motion” and “1, motion”. (c) Trajectory of a triangular swimmer with engineered serpentine microchannels. The continuous and directional motion was marked in blue. (d) Trajectory of a bird-shaped swimmer with engineered straight microchannels engineered on the tail/wings. Inset shows the image sequence of a linear motion of a bird. (e) Velocity characterization of a flat triangular, microchannels engineered triangular and bird shaped swimmers.
1.3.2 External field driven swimmers

Inspired by the motion of a sperm cell and other nature’s swimmers at the microscale, Figure 1-2, Dreyfus et al. engineered a magnetically driven microswimmer [6]. The swimmer consisted of a red blood cell as the head and a chain of superparamagnetic particles as the flexible tail. These particles were connected by streptavidin and biotin interaction as shown in Figure 1-6 (top). As a result, when an external oscillatory magnetic field ($B_x = 8.3$ mT, $B_y = 13.7$ mT at 10 Hz) was applied, the tail vibrates non-reciprocally and induces a motion. The beating pattern of the flexible tail was tracked photographically, as shown in Figure 1-6 (a)-(t). Though, the swimming concept is interesting, repeatable fabrication such as its length may become challenging. Recently, Ghosh et al. demonstrated a controlled and repeatable fabrication procedure of magnetic based swimmer [18]. The swimmer consisted of a helical tail and a spherical head, as shown in Figure 1-7 (top). The dexterity of the swimmer’s motion was shown in Figure 1-7 (middle). Figure 1-7 (bottom) shows the maneuvering of small silica beads by the magnetic swimmers, thus showing the promise of using these swimmers as drug carriers.

Snezhko et al. demonstrated an interesting method to achieve propulsion in the presence of external magnetic field, where any prefabricated swimmer is not needed [10]. They learned that when the ferromagnetic microparticles are placed at the water/air interface, they assemble in magnetic snakes under certain condition of magnetic field strength and frequency. These snakes spontaneously break the symmetry of the surface flow (Figure 1-8 (a)) and start propelling as shown in Figure 1-8 (b). The magnetic snake operates at ~ 100 Hz moves in a straight line, unless collide with the boundary of the
Figure 1-6 Schematic of the magnetic based swimmer (top). (a)- (t) Beating pattern of the flexible tail in an oscillatory magnetic field [6].
Figure 1-7 SEM image of a chiral colloidal swimmer (top). Motion of the swimmer (middle). Maneuvering small silica beads (bottom) [18].

chamber. The oscillation of the snake particles (tail) produced asymmetric circulatory flow pattern, shown in Figure 1-8 (c). In addition, to achieve finer directionality, a large bead (1.5 mm) is placed in front of the assembled snake; Figure 1-8 (d) shows the corresponding motion.
Due to simple operation, magnetically based microswimmers has been extensively studied in the literature. Here I will highlight artificial swimmers that utilize different external fields. Unlike magnetic field driven swimmers, light driven ones has remained relatively unexplored. Camacho-Lopez et al. designed a swimmer that contained liquid crystal doped with azo dye [7]. Azo dye serves to amplify the swimmer’s deformation. The excitation light changed the orientation of the liquid crystal,
which gave rise to internal stresses and the eventual deformation of the swimmer [7]. A fixed swimmer was seen to bend by more than 60°, Figure 1-9 (a). Figure 1-9 (b) shows the dynamics of force generated when visible light was illuminated for 60 milliseconds. To demonstrate propulsion, a circular disc (Ø=5 mm) was placed at the air/water interface, where it propel in the presence of visible light. Figure 1-9 right (a) shows the image sequence of the propulsion. To verify that the propulsion was independent of any surface tension effect, they performed experiments on water containing soap. In both cases, they achieved similar propulsion displacement, depicted in Figure 1-9 right (b). Photons from light produce momentum. Liu et al. fabricated 100 nm sized plasmonic

Figure 1-9 (a) The optomechanical response of the liquid crystal based swimmers. Swimmer’s bending of 60° was achieved by exposing to green light (left). (b) Graphs showing the dynamics of force produced when the visible light was on and after illumination for 60 milliseconds (left). (a) Image sequence demonstrating the propulsion (right). (b) Graph showing the displacement of the swimmer versus time on a clean surface and on a soap surface (right) [7].
Figure 1-10 (a) Nanosize plasmonic motor (b) Electric field and Poynting vector induced at wavelength of 810 nm. (c) Electric field and Poynting vector induced at wavelength of 1700 nm. (d)-(f) Rotation of silica microdisk driven by plasmonic motor [19].

motor (Figure 1-10 (a)-(c)), where they engineered rotational directional by exciting different wavelength (Figure 1-10 (d)-(f)) [19].

Recently, Jiang et al. demonstrated that one can able to propel a swimmer by harnessing thermal energy from the environment [9]. Spherical Janus silica particles were fabricated; with half side coated with gold and the other half remained unexposed; Figure 1-11 (Left) (a)-(b). When laser (ND: YAG $\lambda = 1064$ nm) was excited on the swimmers, the local temperature rises more on the side coated with gold than the silica side, which creates a thermal gradient across the swimmer, thus causing it to propel. Figure 1-11(Left) (d)-(f) shows the swimmer motion in the absence and presence of laser,
Chang et al. demonstrated propulsion of semiconductor diodes in an alternating external electric field [20]. The swimmer produced localized electro-osmotic flow, which allowed them to propel either towards the cathode or the anode, depending on the surface charge. Figure 1-12 Left shows the schematic of propulsion mechanism. Rotational and translation motions were demonstrated, shown in Figure 1-12 Right (a)-(c). Recently, Loget et al. demonstrated propulsion of conducting metallic beads by water splitting via bipolar electrochemistry in the presence of electric fields, the mechanism is shown in Figure 1-13 (a) and (d) [11]. The metallic swimmers triggers oxidation and reduction reactions and generate asymmetric hydrogen and oxygen gas bubbles. The recoiling of gas bubble produced the necessary thrust required to attain propulsion, Figure 1-13 (c)-(f).
Figure 1-12 Left. Schematic diagrams of the experiments. Right (a) Translation motion of a self-propelling diode. (b) Two swimmers move towards the top or bottom depending on the orientation of their anodes (marked with white arrows). (c) Rotation motion [20].

Though electric-field induced propulsion hold promises in particles manipulation and assembles, the field usually requires high applied electric field; not suitable for biological experiments. Low power ultrasound not damaging to biological cells can be utilized in propulsion studies. Wang et al. demonstrated propulsion and assembly of bimetallic rods in the presence of acoustic filed operating at 3.7 MHz, shown in Figure 1-14 (a)-(e) [21]. The motion can be attributed to the asymmetric ultrasound scattering from the edges of the swimmer. Recently, Kagan et al. demonstrated propulsion of cylindrical shaped microswimmers in the presence of ultrasound. The swimmers, which contained perfluorocarbon emulsions was vaporized during ultrasound irradiation, thus producing bullet-like motion, Figure 1-14 (a-b) [22].
Figure 1-13 (a)-(c) Linear motion of spherical objects. (a) Schematic of water splitting by bipolar electrochemistry. (b) Image showing a 1 mm spherical metal particle exposed to 1.6 kVm\(^{-1}\) electric field in sulphuric acid solution. (c) Translation motion of the metallic bead at 5.3 kVm\(^{-1}\). (d) Schematic shows the quenching of oxygen bubble production. (e) Translation motion of a bead at 1.3 kVm\(^{-1}\) [11].

Figure 1-14 (a)-(c) Illustrations demonstrate directional, rotational and assembly of nanorods in the presence of ultrasound [21].
Figure 1-15 (a) Schematic shows the loading of perfluorocarbon within the cylindrical microswimmer. (b) Schematic demonstrates the collapse of perfluorocarbon emulsion in the presence of ultrasound [22].

1.3.3 Applications

The biggest promise of micron sized swimmers lies in performing in vivo experiment, Figure 1-1. However, simple experiment such as noninvasive in vivo drug delivery is still farfetched. Promises in this area were demonstrated in in vitro conditions. Kagan et al. demonstrated the first proof-of-concept work of catalytic motor guided by an external magnetic field to pick up, transport and release drug, schematic shown in Figure 1-16 (a) [23]. The drug was loaded with magnetic particles such that when the catalytic motor comes in close vicinity of the swimmer, the particle get attracted to the nickel region of the swimmer, Figure 1-16 (b). The particle was released when the swimmer makes a fast
turn, during which the drag force becomes larger than the weak magnetic force, Figure 1-16 (c). Recently, Balasubramanian et al. demonstrated specific capture and isolation of circulatory tumor cells by microswimmers, Figure 1-17 [24]. The swimmer was functionalized with anti-CEA mAB-modified, which can detect CEA surface antigens on the target cancer cells, thus allowing selective capture and transport [24], as shown in Figure 1-17 (a)-(b). Xi et al. recently demonstrated self-folded microswimmers with sharp ends can be used as drilling a hole on living tissues [25]. Their swimmers when exposed to rotating magnetic fields change their orientation from horizontal to vertical and started drilling. They have demonstrated successful drilling of approximately 10 micrometer on a dead liver tissue, as shown in Figure 1-18.

Figure 1-16 (a) Schematic showing the pickup, transport and release of drug. (b) Sequential microscope images demonstrated (a) magnetically guided motion of nanomotors towards the magnetic cargo, (b) and (c) demonstrate pick, transport and release of the drug [23].
Figure 1-17 Microswimmer to capture and isolate cancer cells. The swimmer was functionalized with anti-CEA mAB which can detect the CEA surface antigens of the cancer cell (top). (a) Pickup and transport of a CEA + pancreatic cancer cell by an anti-CEA mAB-modified swimmer in PBS and (b) in human serum [24].

Figure 1-18 (a) Microdriller/microswimmer in a rotational magnetic field. (b) Images showing the motion of swimmer under rotating magnetic field of 20 mT. The swimmer moved towards the center of the magnetic field (c) Schematic showing the motion of the swimmer towards the magnetic field and the drilling operation on liver tissue [25].
1.4 Results and discussion

Ultrasound widely used in therapeutics and diagnostics hold plenty of promise in performing non-invasive surgery [26], [27]. However, ultrasound based propulsion is little explored. Here I will discuss the various swimmer designs and concept that I have tried, briefly go through the results, and finally discuss my current acoustic-based swimmer.

1.4.1 Tear drop structure

It is well known that when acoustic waves excited on a solid object submerged in liquid; liquid recirculated around it and produced vortices, a phenomenon known as acoustic streaming. Acoustic streaming around solid objects had been utilized in numerous applications such as in red blood cell lysis [28], chemical reaction [29] and in particle trapping in microfluidics environment [30]. Since acoustic streaming generation around a solid object is primarily depended on the shape of the object and the excited frequency. I have designed and fabricated a tear drop-shaped brass swimmer, as shown in Figure 1-19 (a). Based on the geometry of the structure, the swimmer was expected to have two separate resonance frequencies; circular back end ($f_1$) and sharp front ($f_2$). When the swimmer is excited at frequency $f_1$, circular end generate acoustic streaming and propel. On the other, when the sharp end is excited at frequency $f_2$, it will generate acoustic streaming and propel in the opposite directions, the concept is schematically shown in Figure 1-19 (c). To demonstrate this concept, I have devised a simple experiment. A piezo transducer, which generates acoustic waves, was bonded at the back of a petri dish.
Then the dish was filled with water and the metallic swimmer was carefully placed at the water interface, as shown in Figure 1-19 (b). When the swimmer was acoustically excited, it propelled in a zigzag motion. However, the swimmer propelled only at high acoustic power and did not show bidirectionality.

![Tear drop structure](image)

Figure 1-19 Acoustic based propulsion of a flat tear drop structure. (a) Design of a metallic tear-drop structure. (b) Free-body diagram of the tear drop structure. (c) Bi-directionality concept via acoustically streaming.

1.4.2 Artificial sperm cell

Next we fabricated a sperm-cell shaped swimmer by soft lithography method [17], where the stiffness was similar to that of a red blood cell [31]. Figure 1-20 (a)-(c) shows the micrograph of the swimmer. A swimmer (submerged) was placed in a thin liquid and
acoustic waves were excited. For varying frequency and at maximum applied voltage, the swimmer did not show any sign of propulsion. I believe, the incident acoustic waves from the piezo transducer were absorbed by the soft gel based swimmer. Figure 1-20 (d)-(h) shows a beating pattern of the tail when the water flows past it.

1.4.3 Bubble based swimmer

When acoustic waves interact with an air bubble submerged in liquid, the air bubble oscillates. The amplitude of the bubble oscillation becomes maximum, when the external acoustic waves are excited at the bubble’s natural frequency. Usually a suspending spherical bubble submerged in liquid does not produce any propulsion at low acoustic power [32]. However, we observed that a spherical bubble inside a microfluidic channel generated propulsion at low acoustic power, as shown in Figure 1-21.
Experimentally, I observed that a spherical bubble resting on one sidewall of the microfluidic channel propelled for a short distance at one specific frequency. Consequently, when the acoustic wave frequency was changed continuously from 28.1 kHz to 32.4 kHz, the bubble propelled linearly from right to left, as shown in the image sequence in Figure 1-22 (a)-(e). On the other hand, when the excited frequency was changed from 32.4 kHz to 28.1 kHz, the bubble propelled linearly from left to right, as shown in Figure 1-22 (f)-(j). And when a similar size bubble was immersed in water without any boundary, the bubble didn’t propel.

Inspired by the result shown in Figure 1-22, I ask myself if propulsion is possible in the absence of a wall. To demonstrate this concept I will need a doublet of bubbles. I injected two bubbles of different size by a syringe in an open container holding water with a transducer bonded adjacent to the liquid chamber. The two bubbles did not coalesce, since I have added a little amount of surfactant, known as Tween 20 in water. I observed that at a certain frequency, during acoustic excitation, these two bubbles came together and formed a doublet, Figure 1-23. The doublet was formed due to scattering and interaction of the two bubbles, by a process known as secondary Bjerknes force [33], [34]. At the doublet state i.e. when the bubbles held themselves together, I swept the frequency to 14.5 kHz; the smaller bubble got excited, which was confirmed by large
amplitude oscillation of the smaller bubble under microscopic visualization. Consequently, the surrounding liquid near the smaller bubble was pushed away from it and the doublet started to propel from left to right, as shown in Figure 1-23 (a)-(g). Again, at the doublet state (keeping the excitation acoustic waves on), we decreased the excitation frequency to 9 kHz. This time the bigger bubble got excited. It is already known that the acoustic resonance frequency of a bubble is dependent on its diameter and can be approximated by the Minnaert equation [35]. Consequently, at the larger bubble resonance, the surrounding liquid was pushed away from the larger bubble and the doublet propelled from right to left as shown in Figure 1-24 (a)-(g). Propulsion experiments were carried out while maintaining the acoustic excitation on all time during experiments. At times, I observed the disassociation of the doublet at higher frequencies than those used in the experiment. Furthermore, as the acoustic waves were turned off, the two bubbles disassemble and drifted apart and if the separated distance of the two bubbles were more than ~ 2-3 the diameters of the bigger bubble, it was found difficult to again form a doublet.
Figure 1-22 (a)-(e) Self propulsion of a microbubble from right to left inside a 240 µm width microchannel as the frequency increased from 28.1 kHz to 32.4 kHz. (f)-(j) Self propulsion of a bubble from left to right when frequency decreased from 32.4 kHz to 28.1 kHz.
Figure 1-23 (a)-(g) Image sequence (scale bar 100 µm) shows a doublet moving left to right in the presence of acoustic waves at a frequency of 14.5 kHz and 20V_{pp}.

Figure 1-24 (a)-(g) Image sequence (scale bar 100 µm) shows a doublet moving right to left at an excitation acoustic wave of 9 kHz and 20V_{pp}.
Finally, we developed a swimmer that is neither dependent on the boundary nor depended on association of the two bubbles only in the presence of acoustic waves. The microswimmer I present in this dissertation was conceived outside the scallop theorem design paradigm [16], using reciprocal motion to produce propulsion—and to great effect. Our microswimmer consists of a rectangular polymer body with conical indentations on one side, shown schematically in Figure 1-26 (a). We fabricate the rectangular body and its indentations using a straightforward ultraviolet (UV) photopolymerization method, as shown in Figure 1-25. Indentation diameter was controlled by the photomask, with a small amount of variability introduced by the UV exposure duration, as illustrated in Figure 1-25 (b). Indentation depth was controlled by the UV exposure duration, as illustrated in 1-25 (c) and (d). The exposure time of UV light was optimized to tune the depth of the indents, which facilitated stable and reproducible bubble trapping following a surface treatment. When the microswimmer is submerged in liquid, an air bubble got trapped in each of its indentations.
Figure 1-25 Fabrication and design of the microswimmers. (a) Schematic of the fabrication setup. PEG solution containing photosensitive initiator is sandwiched between glass slides. The swimmers’ geometry and the conical shaped indents were created by exposing the oligomer solution to UV light passing through a transparency mask containing the blueprint of the swimmers. (b) Plot of indentation diameter versus UV exposure time. (c) Plot of indentation depth versus UV exposure time. (d) Images showing the decrease in indentation depth for increasing UV exposure time.

If the liquid surrounding the microswimmer is subjected to an acoustic field, the trapped bubble is driven into oscillations of compression and expansion, tracked photographically in Figure 1-26 (b). When the frequency of the driving acoustic field approaches a resonant frequency of the trapped bubble, the oscillating displacement of the liquid-air interface becomes large, generating a net force along the bubble axis that
Figure 1-26 Swimmer function and experimental setup. (a) Schematic of the swimmer is shown, along with its source of propulsion: an acoustically-driven bubble trapped within the indentation radiates acoustic waves, transferring momentum into the liquid. When the swimmer reaches its terminal velocity, propulsive force \( (F_p) \) is balanced by drag force \( (F_D) \). (b) Image sequence recorded at 360,000 frames per second shows bubble oscillation within the conical indentation. The oscillation displacement is fitted to a sine function (solid line). (c) Schematic of the experimental setup. A piezoelectric transducer, which generates acoustic waves (blue ripples), is placed adjacent to the chamber (filled with water or gel). Boundaries of the chamber were surrounded by acoustically-absorbent putty. Top and bottom were enclosed by glass slides. (d) Fluorescent images of linear swimmers fabricated with single (pseudo colored: yellow) and double (pseudo colored: red) indents symmetric to the center \( y' \) axis. Fluorescent image of a rotational swimmer fabricated with an off-centered indent (pseudo colored: purple). Fluorescent image of a directional swimmer fabricated with different sized indents (green).

propels the microswimmer. To observe and characterize the microswimmer, the experimental setup of Figure 1-26 (c) was devised; acoustic waves are created in the
ambient liquid via a piezoelectric transducer, which is attached to the glass slide and driven by a function generator.

The microswimmer design was characterized with multiple variations of air bubble size, shape, and location. Bubble size and shape were varied because they determine the bubble’s resonant frequencies; to control size and shape, we changed indentation max diameter (50-100 µm), indentation depth, and hydrophobic treatment duration. Bubble location was varied because it determines the swimmer’s type of motion. Figure 1-26 (d) shows the different types of swimmers used. A bubble trapped at the microswimmer center caused translational motion as shown in Figure 1-27 (d), Figure 1-28. On the other hand, a bubble trapped off-center resulted in rotational clockwise motion (Figure 1-29 (a)); swimmers with off-centered indents on the opposite side rotate anticlockwise (Figure 1-29 (b)). Furthermore, two bubbles (of equal size) each trapped off-center caused counteracting moments, resulting in translational motion (Figure 1-27 (e)). Swimmers are propelled autonomously in the path defined by their initial orientation and with a direction opposite the exposed bubble, Figure 1-26 (a). The force/moment created by these acoustically-driven bubbles was tuned by adjusting the intensity of the applied acoustic field (i.e., the voltage applied to the piezoelectric transducer)—the higher the voltage, the larger the force/moment. Experiments demonstrate that this force/moment can be made very high, achieving fast swimmer movement in even highly viscous liquids, as shown in Figure 1-29 (c) and Figure 1-30 (a)-(g). Translational velocities up to 8 mm/sec (~50 body lengths per second) and rotational velocities up to 1150 rpm were observed in water, and translational velocities up to 50 µm/sec and rotational velocities up to 3 rpm were observed in viscous hydrogel.
Figure 1-27 Translational motion. (d) Image sequence recorded at 30 frames per second demonstrates linear motion a single bubble at placed at the center. (e) Image sequence recorded at 10,000 frames per second demonstrates translational motion, moving left to right, of a swimmer in water/bead solutions.

Figure 1-28 Translational motion of linear swimmers.
So how does the reciprocal motion of an oscillating bubble—driven by an acoustic field—produce a net propulsive force? First, note that the wavelength of applied acoustic wave ($\lambda \sim 14$mm) was much larger than the microswimmer dimension, meaning the swimmer is subjected to essentially equal liquid pressure on all its surfaces at any given point in time. We confirmed this experimentally by exposing bubble-less microswimmers to an acoustic field of varying frequency and amplitude; the swimmers did not move, Figure 1-31 (a)-(b).

So why does the bubble-containing microswimmer propel in an acoustic field, even though the air-liquid interface (bubble) undergoes cyclic reciprocal motion? To gain some intuition on the governing physics, we consider the air-liquid system to be a spring-

Figure 1-29 Rotational motion. (a)-(c). High speed recordings of off-centered swimmers in water/bead solutions captured at 10,000 frames per seconds demonstrate (a) clockwise motion (marked yellow) (b) anticlockwise motion (marked red) with an indent designed on the opposite. (c) Rotational motion of swimmers in viscous gel captured at 16 frames demonstrates clockwise motion (marked blue) and anticlockwise motion (marked magenta).
mass damped forced oscillator, where the mass consists of the liquid in the immediate vicinity of the air bubble, and the spring consists of the air. As the bubble oscillates, the energy is transferred between kinetic energy of the liquid mass and potential energy of the air spring. Energy crosses the boundary of this system in two ways: the driving force, and damping. The driving force is provided—by the incident acoustic wave transferred from the piezotransducer. This force can act either in the direction of the liquid mass’s movement—adding energy to the system—or opposite the direction of the liquid mass’s movement—removing energy from the system. Damping serves only to remove energy.
from the system, and comes in the form of radiation, thermal, and viscous damping; thermal and viscous damping are both small relative to radiation damping [35].

Figure 1-31 (a) Linear motor with no bubble trapped was placed in water containing bead solutions. (b) Rotational motor with no bubble trapped was located in viscous gel floating in a three dimensional space.

Radiation damping, which is responsible for propulsion, is maximized when the frequency of the driving force equals the resonant frequency of the air-liquid (spring-mass) system. At resonance the bubble oscillations provides efficient localization of acoustic energy i.e. the secondary acoustic wave is intensified. The ratio of secondary to incident wave intensities can be estimated by the following expression [35]

\[
\frac{\Omega^{\text{scat}}}{\pi R_0^2} = \frac{4}{\left(\frac{\omega_0}{\omega}\right)^2 - 1}\left(\frac{2\pi R_0}{\lambda}\right) \ll 1 \quad \text{(Eq. 1)}
\]

where \( R_0 \) is the bubble equilibrium radius, \( \omega_0 \) is the bubble angular resonance frequency, and \( \omega \) is the incident angular frequency and \( Q \sim 25 \) is the experimentally determined quality factor, Figure 1-32, of the bubble oscillation. Therefore, at resonance frequency (\( \omega = \omega_0 \)), the secondary acoustic wave is intensified by three order of magnitudes \( \left(\frac{\Omega^{\text{scat}}}{\pi R_0^2} = 4Q^2 \sim 2500\right) \). Focused energy from radiation damping reradiates
away axially from the bubble through maximum oscillations of its liquid-air interface in the form of secondary acoustic wave in the liquid.

The propagation of secondary acoustic waves generates localized radiation pressure, which in turn generate a propulsive force. Here we present a theoretical treatment of this force and show how the velocity of our microswimmer can be controlled by adjusting voltage amplitude of the signal generator. The increment of the localized pressure $P_{ave}$ compared to the environmental $P_0$, can be expressed as [36]:

$$P_{ave} - P_0 = \frac{1}{2} \rho \omega^2 \varepsilon_0^2$$  \hspace{1cm} (Eq. 2)

where $\rho$ is mean density of water, $\omega$ is angular frequency of the wave (equal to the driving signal angular frequency), and $\varepsilon_0$ is displacement amplitude of the wave (equal to displacement amplitude of the bubble oscillation). The pressure increase near the cyclic
reciprocal motion of the bubble was *visualized experimentally* by fixing a microswimmer in place, and introducing small particles to the liquid (these particles move with the liquid due to drag forces), showing movement of a particle (along the bubble axis) that is periodically away from the bubble and then towards the bubble—but on average is away from the bubble, thus indicating a net force, as shown in Figure 1-33. This force being applied by the bubble on the liquid—and, by Newton’s third law, the force being applied by the bubble on the microswimmer—is $\propto \varepsilon_0^2$. Experimental results demonstrate a clear linear relationship between $\varepsilon_0$ and input signal voltage amplitude $V$, with input signal frequency held constant, Figure 1-34. Therefore, propulsive force is proportional to the square of the input voltage, $F_p \propto V^2$.

This correlation between the bubble amplitude oscillation $\varepsilon$ and the voltage applied $V$ via a piezo transducer was verified. Experiments were performed by fixing the microswimmer in place, with its bubble driven at a resonance frequency. Then the voltage applied was increased by 1 volt and the corresponding oscillation response was
recorded by a fast camera. Figure 1-34 shows the linear relation between voltages applied \( V \) and bubble oscillation amplitude \( \varepsilon \).

![Graph showing linear relation between voltage and bubble oscillation amplitude](image)

Figure 1-34 Empirical data showing linear relation between input signal voltage and bubble oscillation amplitude (with driving frequency held constant).

**Velocity characterization of the microswimmer in water**

As the swimmer moves through the liquid, drag force is developed. At low Reynolds number \((Re < 0.9)\), the Stokes’ drag is linearly proportional to the swimmer velocity, \( F_D \propto v_{\text{swimmer}} \). Experiments indicate that as the input signal frequency approaches a bubble resonant frequency, the swimmer begins to move, and it quickly reaches terminal velocity, at which \( F_p = F_D \). Therefore, the velocity of the microswimmer can be controlled via the input voltage through the relation \( v_{\text{swimmer}} \propto V^{2.0} \); this relation was demonstrated empirically with microswimmer travel through water, as shown in Figure 1-35. Though the data demonstrated good correspondence with theory \((v_{\text{swimmer}} \propto V^{2.1 \pm 0.1})\), we recognized that the complex circulatory flow pattern or more accurately
Figure 1-35 Characterization of the swimmers. Mean swimming velocity \( \nu \) of the swimmers versus driving voltage \( V \) in water/bead solutions. The driving voltage \( V \) is directly proportional to the amplitude oscillation \( \varepsilon \) of the bubble. Dashed line (magenta) represents the quadratic fit, with \( \nu \propto \varepsilon^2 \). Inset shows the slope of 2.1 ± 0.1 drawn as a solid line (magenta).

Figure 1-36 Acoustic driving of the microswimmer’s bubbles generates substantial acoustic streaming in water.
acoustic streaming developed near the oscillating bubble in water may be affecting drag along the swimmer body (see Figure 1-36).

**Velocity characterization of the microswimmer in hydrogel**

![Figure 1-37](image)

- (a) Swimmers immersed in viscous liquid in the absence of acoustic waves. (b). No streaming was observed in the presence of acoustic waves. 4 \( \mu \)m tracer particles were seen to oscillate close to the bubble surfaces.

We conducted microswimmer characterization in viscous hydrogel (in which the swimmer had \( Re < 2.1 \times 10^{-6} \), in which viscous dissipation caused acoustic streaming patterns to be highly localized near the bubble, ensuring no effect on drag, Figure 1-37. The gel shows shear thinning behavior, as shown in Figure 1-38, which is accounted for in the drag force equation via experimentally-determined parameters \( K, n, \) and \( Y \) \[^{[37]}\]

\[
F_D = 3\pi K n d^2 \phi^{2-n} c_d Y
\]  
(Eq. 3)

The flow behavior index \( n \) was measured as 0.49, such that \( F_D \propto \nu_{swimmer}^{0.49} \). Thus, equating \( F_P \) and \( F_D \) in the gel yields the swimmers' velocity as a function of bubble oscillation amplitude, \( \nu_{swimmer} \propto \epsilon^{2/n} \) or \( \nu_{swimmer} \propto \epsilon^{4.0} \). This power law relation was
Figure 1-38 Viscosity $\mu$ (solid black) and shear stress $\tau$ (dashed blue) of the shear thinning gel versus shear rate $\gamma$. Flow consistency index $K$ and flow behavior index $n$ are experimentally measured as 9.2 and 0.49 for the power-law fluid.

Figure 1-39 Mean swimmers velocity with 30 $\mu$m (solid red) and 50 $\mu$m (solid blue) bubbles trapped versus bubble oscillation amplitude $\varepsilon$. Inset provides the slopes of $4.0 \pm 0.2$ and $4.0 \pm 0.1$, respectively, drawn as dotted lines (blue and red) suggest a power law relationship between $v$ and $\varepsilon$, where $v$ increases as $\varepsilon^n$. 
demonstrated empirically using two different swimmers in Figure 1-39, showing excellent correspondence with theory ($v_{\text{swimmer}} \propto V_0^{4.0 \pm 0.2}$).

**Generation of acoustic radiation pressure**

In this section, we try to understand how the pressure is generated from an oscillating bubble to the surrounding liquid. The bubble is driven into oscillation of compression and rarefaction, which acts as the sole source of acoustic waves since its energy is intensified by three orders of magnitudes. Considering a small liquid volume near the bubble, where it moves back and forth in phase with the bubble oscillations and it gains velocity only in the direction of the wave propagation. To understand this pressure developed we introduced an imaginary piston positioned axially to the opening of the oscillating bubble (arrangement shown in Figure 1-40). The wave propagation in the $x$ direction causes the stress developed in the liquid, which can be described by the Brillouin’s stress tensor [36]

\[
\begin{pmatrix}
-(p) - \langle \rho v_x^2 \rangle & 0 & 0 \\
0 & -(p) & 0 \\
0 & 0 & -(p)
\end{pmatrix}
\]  
(Eq. 4)

where $\langle \rho v_x^2 \rangle$ is equal to the acoustic energy density $E$, and is equal to $\frac{1}{2} \rho \omega^2 \epsilon^2$, and $(p)$ is the isotropic stress (acting along the $x$, $y$, and $z$ direction). Note the term $\langle \rho v_x^2 \rangle$ in the tensor expression is responsible for the pressure increase in the $x$ direction, *i.e.*, the stress developed by the oscillating bubble, which is always larger than the stress developed in the $y$ and $z$ direction by a quantity of magnitude $E$. 

Figure 1-40 contains two pistons: one facing the bubble while the other is placed perpendicularly (parallel to positive y direction). In our model, we assume both pistons are perfect acoustic absorber and are fixed. When the bubble start to oscillate, it generate acoustic waves, consequently increases the pressure of piston 1 (x direction) by $P'_{ave}$, and using stress tensor from eq. 4 we can estimate the pressure developed in piston 2 (acting in the y direction), which is $(P'_{ave} - E)$. The modified tensor was shown below:

$$-egin{pmatrix} P'_{ave} & 0 & 0 \\ 0 & P'_{ave} - E & 0 \\ 0 & 0 & P'_{ave} - E \end{pmatrix}$$

(Eq. 5)

Now, we have released piston 2, which is shown in Figure 1-40 (b). As a result, the pressure difference between the top $(P'_{ave} - E)$ and bottom ($P_0$, environmental pressure) of piston 2 will push piston 2 upward until the pressure difference become zero, as a result the new top pressure of piston 2 becomes $P_0$. As mentioned above, the pressure developed in the x direction is larger than the pressure developed in the y and z direction by $E$, thus the new left pressure of piston 1 is $P_0 + E$, which is the pressure developed by an oscillating bubble.

$$-egin{pmatrix} P_0 + E & 0 & 0 \\ 0 & P_0 & 0 \\ 0 & 0 & P_0 \end{pmatrix}$$

(Eq. 6)
Propulsive force acting on microswimmer

The propulsive force acting on the swimmer is equal to the force being generated by the bubble on the liquid. To estimate this, we examine the time-averaged pressure created by the bubble in the liquid (Eq. 2), and integrate this over the bubble surface. The distortion of the bubble’s liquid-air interface can be complex at higher harmonics, but the first harmonic shape was observed to be always a simple outward bowing. This outward bowing is shown in the sketch below, Figure 1-41, and its shape matches well to a cosine function. Therefore, we approximate the displacement amplitude of the bubble surface...
from its undisturbed position, $\varepsilon_0$, as a function of distance $r$ from the bubble axis, as follows: $\varepsilon_0(r) = \sigma_0 \cos\left(\frac{\pi}{2R} r\right)$.

![Figure 1-41 Model to approximate bubble oscillatory displacement across its surface area.](image)

Using this approximation for $\varepsilon_0$, we examine the time-averaged pressure created by the bubble in the liquid, and consider its action on a finite 3D surface area element $r \cdot dr \cdot d\theta$.

$$F_P = \int_0^{2\pi} \int_0^R \frac{1}{2} \rho \omega^2 [\varepsilon_0(r)]^2 r \, dr \, d\theta = \pi \rho \omega^2 \sigma_0^2 R^2 (0.25 - 1/\pi) \quad (\text{Eq. 7})$$

where $\rho = 1000 \text{ kg/m}^3$ is the mean water density, $\omega = 2\pi f$ ($f = 94.4 \text{ kHz}$ for small swimmer and $f = 70.4 \text{ kHz}$ for big swimmer) is the angular frequency of the bubble-
generated acoustic wave (which is equal to the angular frequency of the driving acoustic wave), $\sigma_0$ is the maximum value of the bubble displacement amplitude $\varepsilon_0$ (~7 µm for small swimmer and ~10 µm for big swimmer), and $R$ (15 µm for small swimmer and 33.3 µm for big swimmer) is the radius of the liquid-air interface.

**Drag force acting on microswimmer**

When an acoustic wave is applied to the ambient liquid, the swimmer accelerates and very quickly reaches terminal velocity, at which the propulsive force is counteracted by an equivalent drag force ($F_p = F_D$). The drag force acting on the swimmer can be computed by:

$$F_D = 3\pi \mu v_d v C_d$$  \hspace{1cm} (Eq. 8)

where $\mu$ is dynamic viscosity of the liquid, $v$ is the swimmer velocity, $d_v$ (118 µm for small swimmer and 250 µm for big swimmer) is the width of the swimmer, and $C_d$ is the dynamic shape factor. Because the viscous gel exhibits shear thinning, the dynamic viscosity $\mu$ in the drag force equation is replaced by the effective viscosity $\mu_e \approx K(v/d_v)^{n-1}$. The power-law model for effective viscosity is simplistic, and a coefficient $Y$, with a value on the order of unity, is often used to bring the results of the model more in line with observed viscosity ($\mu_e \approx K\left(\frac{v}{d_v}\right)^{n-1}Y$). So drag force counteracting swimmer movement in the gel can be computed as:

$$F_D = 3\pi K v^n d_v^{2-n} C_d Y$$  \hspace{1cm} (Eq. 9)

**Force characterization of the microswimmer in hydrogel**
We reinforced our theoretical treatment by calculating the propulsive force $F_p$ for the two gel swimmers for various values of bubble amplitude, Figure 1-42. Using the associated measured terminal velocities of Figure 1-39, we calculated the drag $F_D$; this result included an unknown parameter $C_d \cdot Y$, which should be on the order of unity. By adjusting the $C_d \cdot Y$ parameter until the calculated drag force coincided with the calculated propulsive force, we found $C_d \cdot Y$ parameters of 2.2 and 4.2 for the two swimmers, which coincide very well with the expected value of unity.

![Figure 1-42](image.png)

**Figure 1-42** Acoustic propulsive force $F_p$ (red circles) and drag force $F_D$ (green circles) is plotted as a function of bubble oscillation $\varepsilon$. Dashed line (green) is a fit to a quadratic dependence on the swimmer $F_D$ and $\varepsilon$, $F_D \propto \varepsilon^{1.94}$.

**Reynolds number of the microswimmer**
In water, $0.075 < \text{Re} = \frac{ud_v}{\theta} < 0.9$, where $500 < u < 6000 \, \mu\text{m/s}$ is the observed speed of the swimmer, $d_v \approx 150 \, \mu\text{m}$ is the width of the swimmer, and $\theta \approx 1.0 \times 10^{-6} \, \text{m}^2/\text{s}$ is the kinematic viscosity.

In viscous gel, $\text{Re} = \frac{ud_v}{\theta} < 2.1 \times 10^{-6}$, where $u \approx 50 \, \mu\text{m/s}$ is the maximum observed speed of the swimmer, $d_v \approx 250 \, \mu\text{m}$ is the width of the swimmer, and $\theta \approx 0.006 \, \text{m}^2/\text{s}$ is the kinematic viscosity.

**Reynolds number of the oscillating bubble**

In water, $\text{Re}_{ob} = 4\pi a f \varepsilon / \theta \approx 90$, where $a \sim 30 \, \mu\text{m}$ is the bubble radius, $f = 48 \, \text{kHz}$ is the excited frequency, and $\varepsilon \sim 5 \, \mu\text{m}$ is the maximum amplitude displacement.

In viscous gel, $\text{Re}_{ob} = 4\pi a f \varepsilon / \theta \approx 0.02$, where $a \sim 20 \, \mu\text{m}$ is the bubble radius, $f = 70 \, \text{kHz}$ is the excited frequency, and $\varepsilon \sim 7 \, \mu\text{m}$ is the maximum amplitude displacement.

**Selective actuation of the microswimmer**

Now that we have characterized the swimmer physics and method of control, we progress to describing the most exciting feature of our microswimmer design: it enables, for the first time, the *selective actuation* of one microswimmer from within a group. When a bubble is driven at a frequency dissimilar from its resonance frequency, the displacement amplitude of its vibrations will be very small and the propulsive force it generates will be negligible. When the driving frequency is close to the bubble’s resonance frequency, the resulting propulsive force generates meaningful movement, with the most substantial movement generated at the resonance frequency. Thus, if we have a group of microswimmers $(1, 2, \ldots, n)$, each with a bubble of different size/shape from the others
such that each swimmer has a different first resonance frequency \((\omega_1, \omega_2, \ldots, \omega_n)\), then we can tune the frequency of our driving signal to one of \(\omega_1, \omega_2, \ldots, \omega_n\), selectively actuating one swimmer while the other swimmers remain stationary (or very nearly stationary). To demonstrate this, we fabricated two single-bubble microswimmers of different bubble size, exposed them to an acoustic field, and performed a frequency sweep. The larger bubble reached resonance first, causing translation through the liquid (with \(v_{\text{swimmer}} \propto \sqrt{V_0}\)) while the driving frequency was maintained (Figure 1-43(a)). The driving frequency was then increased until the smaller bubble reached resonance, causing

![Figure 1-43 Selective actuation of a swimmer from within a group. Two swimmers, each with a bubble of different size, were exposed to the same acoustic field, and a frequency sweep was performed. (a) Swimmer A starts to move substantially at an excitation frequency of 74 kHz, with minimal motion of swimmer B. (b) The frequency is then gradually increased, stopping movement of swimmer A. At an excitation frequency of 91 kHz, swimmer B starts to move while swimmer A remains stationary.](image)
translation of the small-bubble swimmer while the large-bubble swimmer remained stationary, Figure 1-43 (b). This establishes the capability for selective actuation of a single acoustic bubble-driven microswimmer from within a group.

**Directionality**

Note that we have so far demonstrated translational motion and rotational motion in separate swimmer designs. But any artificial microswimmer of practical worth should be capable of *both* translation and rotation (*i.e.*, 2D motion). To this end, the concept of varying bubble resonances that permitted selective actuation can also be used to construct a single swimmer that *moves in 2D*. To demonstrate this, we fabricated a swimmer with two off-centre bubbles of different size/shape, with initial resonance frequencies $\omega_a$ and $\omega_b$, as shown in Figure 1-44. The resonance frequencies were sufficiently similar that when an intermediate driving frequency (*i.e.*, $f \approx (\omega_a + \omega_b)/2$) was applied, both bubbles generated approximately equal propulsive force and the swimmer translated, as shown in Figure 1-46, but when a driving frequency equal to either $\omega_a$ or $\omega_b$ was applied, one bubble generated propulsive force sufficiently larger than the other and the swimmer rotated, as shown in Figure 1-45. This 2D motion of a two-bubble swimmer is shown in Figure 1-33, which also highlights the increase in rotational radius (relative to a
Figure 1-44 (a)-(b) Schematic showing directional motion: right and left motion. (c) Plot demonstrating oscillation amplitude of two bubbles versus varying frequency.

Figure 1-45 2D motion of a two-bubble swimmer. (a) A two-bubble swimmer, with bubbles of different sizes, is seen to accomplish rotation by driving one of the bubbles at its resonance frequency; because the other bubble still generates some propulsive force, the two-bubble swimmer has a larger turning radius than the one-bubble swimmer, shown at the centre of the image.
Figure 1-46 (a) Schematic demonstrating linear motion. (b) A two-bubble swimmer, with bubbles of different sizes, is seen to accomplish translation by driving the bubbles at a frequency intermediate between their respective resonances. (b) One-bubble swimmer) due to the presence of a non-zero—but still low—propulsive force generated by the opposite bubble.

**Interaction of multiple swimmers**

Finally, we studied the interactions of multiple swimmers. Figure 1-47 (a) shows the motion of two swimmers placed in water. As expected due to the asymmetric nature of
the swimmers, they perform rotational motions till they approach each other. In this case, the swimmer in the left was influenced by the swimmer on the right, which was due to the interactions of waves generated from the oscillating bubbles. Consequently, they were attracted towards each other and assembled into a single unit; see Figure 1-47 (a). Next we placed symmetric (linear) swimmers in the liquid chamber and due to theirs bubbles’ interactions, the swimmers were first aligned themselves and then merge into a single unit. While maintaining the exact frequency, the assembled swimmers undergo rotation at 1500 rpm, shown in Figure 1-47 (b). On the other when experiments were performed in viscous gel, the two swimmers involved in a hit-and-run situation Figure 1-47 (c), where one swimmer path was not affected by the other swimmer through either attraction or repulsion. This can be attributed due to higher viscous dissipation of acoustic waves from

Figure 1-47 Interaction of multiple swimmers in different liquids. (a) Clockwise motion of two asymmetric one-bubble swimmers in water, which was later seen to translate, come into contact and firmly lock themselves. (b) Clockwise motion of two assembled symmetric (linear) swimmers in water. (c) Two asymmetric swimmers in a hit-and-run situation in a viscous gel. The trajectories of two swimmers before and after the impact were shown by the magenta and the green coloured trackers.
oscillating bubbles in the gel. Experiments based on the liquid properties and swimmer designs will aid advanced collaborative functionalities, which is a necessary and important step towards the development of micro and nano swimmers.

1.5 Future works

1. The developed swimmer can potentially be utilized into a three dimensional swimmer, however much work is required. As a proof of concept, I placed an asymmetric swimmer in viscous gel. Once acoustic waves were applied at the bubble’s resonance frequency, the swimmer started to rotate in out-of-plane motion, as shown in Figure 1-48. Though two dimensional motions have been schematically and experimentally demonstrated, three dimensional motions is challenging and require out of plane indents to trap bubbles. Though, we have fabricated indents that are perpendicular to each, the procedure is time consuming and requires high experimental dexterity.

2. Swimmers holding different sized bubbles can be actuated based on the resonance frequencies of the bubbles trapped. We can move each swimmer by exciting different frequency of acoustic waves. By simply maneuvering each swimmer, we can perform collaborative work. A rudimentary collaborative work is shown in Figure 1-49, where the two swimmers moved a long and slender wire in blood as the surrounding environment.
3. For oil recovery or in applications that require swimmer needs to move through openings, whose dimension is less than the swimmer cross-section dimensions? I fabricated swimmer, whose stiffness is similar to red blood cell [31]. The intended future work is schematically shown in Figure 1-50

![Figure 1-49 Collaborative concept with artificial swimmer](image)

Figure 1-50 Swimmer passes through a narrow constriction. Inset show squishy particles being pushed out from narrow channel [31].
4. Currently I have been working on drug delivery using our microswimmers. To demonstrate this proof of concept, the swimmers were fabricated, washed and then dried. Then the swimmers were loaded with fluorescein dye and washed twice with water to remove the excess dye. Then a swimmer was placed in water and the release of the fluorescein molecules from the swimmer was characterized over time, which is shown in Figure 1-51 (a)-(c).

![Figure 1-51 (a)-(b) Fluorescence loaded swimmers. (c) Characterization of fluorescence (drug) release over time](image)

### 1.6 Conclusion

In the field of microswimmer design, the acoustic bubble-driven microswimmer that we have presented achieves distinguishing performance. The acoustic field that actuates the swimmer is inexhaustible and unaffected by ambient liquid chemistry, making it preferable to chemical actuation techniques. More importantly, a low-power acoustic
field—which is benign to biological systems—still generates enough propulsive force to move the swimmer through even highly viscous fluids. This means our swimmer design could quite realistically be used inside human vasculature, unlike electric and magnetic field actuation techniques (which, to our knowledge, would require biologically-damaging field strength to achieve adequate propulsion). Although our swimmer outperforms existing designs along conventional metrics, we believe the biggest contribution of our design is the demonstration of selective actuation of a single swimmer from among a group—a first in the field. Such selective actuation opens the new possibility of cooperative action among microswimmers. For example, we envision a small collection of two-bubble microswimmers, each capable of being selectively actuated in 2D, and with bubble surfaces coated in polymer to ensure long-term bubble stability, being deployed in vasculature, with actuation provided by an exogenous transducer applied to the skin, and tracked by ultrasonic imaging or magnetic resonance imaging (MRI).

1.7 Methods

Materials

The microswimmers were fabricated using a mixture of photo-crosslinkable polyethylene glycol (PEG) and a photo-initiator. Specifically, the mixture consisted of 40% (v/v) PEG diacrylate with a molecular weight of 700 (PEG(700)DA, from Sigma-Aldrich), 25% (v/v) PEG with a molecular weight of 258 (PEG 258, from Sigma-Aldrich), 15% (v/v)
photo-initiator 2-Hydroxy-2-methyl-1-phenyl-propan-1-one (Darocur 1173, from Ciba), 15% (v/v) TE buffer (100 TE, from OmniPur), and 5% (v/v) fluorescein.

**UV photopolymerization setup**

The PEG/photo-initiator mixture was sandwiched between two glass slides; these slides were coated in PDMS in order to enable easy removal of the swimmer bodies once cured. The two slides were separated by 150 or 250 µm spacers, which determined the length of the swimmer body. The PDMS-coated glass slide was fabricated by spin coating a 10:1 mixture of PDMS:curing agent at 4000 rpm, then curing the polymer at 65°C for 24 hours. Photomasks of different geometries were designed using AutoCAD software and printed at 20,000-dpi resolution (CAD/Art Services, California). The mask was then inserted to the field stop of an inverted microscope (Nikon TE-2000U). A mercury lamp was used as the UV light source. A filter cube (11000v2: UV, Chroma) was used to select light of 373 µm wavelength. A shutter system, controlled by NIS software, was used to control duration of UV exposure.

**Swimmer isolation**

After UV exposure caused selective hardening of the liquid PEG polymer, the hardened polymer bodies of the microswimmers had to be separated from the surrounding liquid polymer. To accomplish this, the swimmers were washed three times in ethanol solution containing 0.05% Tween 20 (from Sigma Aldrich), removing any liquid PEG residue from the hardened PEG surfaces (including from the indentation). Figure 1-35 shows an image of collection of swimmers.
Figure 1-52 Microscopic image (x-y plane) showing multiple swimmers, indicating the high throughput of the UV photopolymerization method.

**Trapping of the bubble**

A drop of ethanol solution containing the microswimmers was placed onto the glass slide used for observations under the microscope. This glass slide was then placed in an oven for 30 minutes at 65°C in order to dehydrate the swimmers. The slide was then moved to a vacuum chamber, where the swimmers were treated with 1H, 1H, 2H, 2H-perfluorooctyl-trichlorosilane for 20–30 minutes to make their surfaces hydrophobic. After this hydrophobicity treatment, drops of liquid (either water or viscous hydrogel) were added to the microswimmers, causing air bubbles to be trapped in the swimmer indentations. The size of the trapped air bubble was a function of the indentation max diameter, the indentation depth, and the hydrophobicity treatment duration.

**Experimental setup for microswimmer characterization**
The glass platform holding the microswimmers in ambient liquid (see Section 1.4) was either a rectangular glass slide (6.08 cm × 2.54 cm) or a circular petri dish (9 or 18 cm diameter). The liquid was bounded on the perimeter by an acoustically absorbent putty, and on the top surface by a glass cover slip. Acoustic waves were introduced to the liquid via the glass slide/petri dish, to which was bonded a piezoelectric transducer driven by a function generator (Tektronix AFG 3011). The glass slide/petri dish was mounted on the stage of a Nikon TE-2000U optical microscope.

**Imaging and tracking**

Microswimmer motion was captured using a Photron SA4 fast camera, connected to the microscope. Raw high-speed images were analysed using NIS tracking software to determine parameters such as translational/rotational velocity.

**Control**

To verify that a bubble-less swimmer does not propel, we carried out couple of control experiments. We placed a linear motor (no bubble trapped) in water/bead solutions, shown in Figure 1-36 (a), then the frequency of the acoustic waves was swept from 20–120 kHz at the highest voltage applied (20 V_{PP}), and no motion was observed. To make sure that the swimmer was not attached to the substrate, we placed a rotational motor (no bubble trapped) but this time in viscous gel, shown in Figure 1-36 (b) (floating in a three-dimensional space). The frequency of the acoustic waves was swept from 20 to 120 kHz at 20 V_{PP}, and again no motion was observed.
References


Chapter 2

Tunable, pulsatile chemical gradient generation via acoustically driven oscillating bubbles

We present a novel concept of using acoustically activated bubbles, designed in ladder-like arrangements to generate both static and pulsatile chemical gradients. Furthermore, by regulating the amplitude of the bubble oscillation, we demonstrate that the chemical gradient profiles can be effectively tuned.

2.1 Introduction

Generating pulsatile chemical gradients in microfluidic devices has important implications for the characterization of dynamic biological [1–4] and chemical processes [5]. Several recent studies have shown that both spatial and temporal characteristics of chemical stimuli play an important role in cell signaling [6–9]. In addition, pulsatile chemical gradients can be useful for high-throughput characterization of cellular processes such as directed migration [10], differentiation [11,12] and apoptosis [13]. Existing techniques to generate chemical gradients include the Boyden chamber and its derivatives [14–16], the micropipette method [17], and microfluidic-based systems [18–22]. Although all of these methods are capable of generating linear and radial chemical gradient profiles in a static manner, limited research [23–27] has been conducted on dynamic temporal control of chemical gradients.
2.2 Results and discussion

In this chapter, we demonstrate that multiple bubbles that are arranged in a ladder-like formation and oscillating in an acoustic field provide a unique and versatile method to generate dynamic chemical gradients in microdevices. Each oscillating bubble, as shown in Figure 2-1 (a), when activated, mixes the stimulus and buffer solutions locally, effectively diluting the stimulant concentration. Subsequent transport of this mixed stimulant to the next bubble in the ladder results in further dilution of the stimulant, thereby generating a spatial gradient of the stimulant across the microchannel. In addition, each of the oscillating bubbles can be turned on or off almost instantaneously, facilitating the generation of pulsatile chemical gradients. Furthermore, by controlling the mixing ratio of the stimulant and the buffer, we demonstrate that the chemical gradient profiles can be tuned on-the-fly.

Our experimental setup (Figure 2-1 (a)) contains multiple bubbles trapped by horseshoe structures. Briefly, a single-layer PDMS channel containing multiple horseshoe structures is bonded to a glass slide, while a piezoelectric transducer is attached adjacent to the channel. After the bubbles were trapped in the horseshoe structures, stimulant (green, Figure 2-1(a)) at the maximum concentration of $C_0$ was introduced into the channel through the right inlet, while the buffer was infused through the left inlet. Parallel laminar flows of stimulant and buffer across the channel were established. When the bubbles were acoustically activated via the piezoelectric transducer, the oscillations of the bubbles exhibited the "microstreaming" phenomenon [28–37] All the horseshoe structures were designed to be of identical geometry, so that
all the trapped bubbles oscillate at a single resonance frequency. In principle, the acoustic microstreaming is generated due to the nonlinear effects of the oscillatory fluid motion produced by the acoustic waves [38]. The pressure and velocity fluctuations in the liquid close to the bubble cause rapid and homogeneous sideward mixing of the co-flowing liquids. As shown in Fig.2-1 (a), at $t_1$, the stimulant and buffer are mixed by the oscillating bubble closest to the inlets, resulting in a lower concentration of $C_1$ as the stimulant approaches the second bubble. At $t_2$, the liquid (after passing the first horseshoe structure) with concentration $C_1$ is mixed with the buffer in the laminar region resulting in further lower concentration, $C_2$. As this step-wise dilution of the stimulant progresses, all the liquid is mixed and merged across the channel resulting in a spatial chemical gradient.

![Figure 2-1](image)

Figure 2-1 (a) Schematic of the experimental setup, a piezoelectrical transducer is placed adjacent to the microfluidic device. An exponential gradient profile is established between the co-flowing
stimulant and buffer in the microfluidic channel. (b) Image sequence acquired at 200,000 fps to capture one complete cycle of an oscillating bubble trapped within a horseshoe structure.

Oscillation of bubbles can be tuned directly by controlling the voltage fed into the transducer. As indicated in the experimental results shown in Figure 2-2 (a), the oscillation amplitude responds linearly to the applied voltage in our experiments. As a consequence, the mixing distance, $d$, varies linearly with increasing applied voltage (Figure 2-2 (a)). Since bubbles trapped within the horseshoe structures are organized in a ladder-like formation with each one offsetting from the last one by a length, $l$, different applied voltage allows different mixing distance, enabling us to achieve different chemical profiles. For a ladder-like horseshoe structure formation used in this article (detailed design given in Figs. S1–S3 of the Supporting Information), we simulated the generated gradient profiles at different mixing distances using a home-made MATLAB code. The code considers both diffusion and bubble-enabled mixing effects, along with the assumptions of homogeneous mixing, no crosstalk between bubbles, and uniform flow velocity along X coordinate. The simulated results for mixing distances of 250 μm and 375 μm are shown in Figure 2-2 (c) and (d), respectively. The simulated chemical gradient profiles measured at downstream (noted by the dashed line in Figure 2-2 (c)) at mixing distance ranging from 250 μm to 600 μm are summarised in Figure 2-2 (b). Clearly we can observe the relationship between the shape of generated gradient profile and the mixing distance that is controlled by the applied voltage. As a result, we can dynamically control the spatial and temporal chemical gradient profile by adjusting the voltage applied.
Figure 2.2  (a) Mixing distance, $d$ of FITC-dextran and PBS as a function of applied voltage ($d$ is experimentally measured from the centre of the horseshoe structure to the region where no mixing occurs).  (b) Simulation results show the dynamic gradient profiles generated when the mixing distance is varied.  (c) and (d) Simulation results show the generation of chemical gradients with different mixing distance ($d=250$ µm and 375 µm, respectively) within the channel.

To experimentally prove the effectiveness of our method, we used Dextran-FITC (stimulant) and phosphate buffered saline (buffer) solutions to generate different spatial
and temporal concentration profiles across the microfluidic channel. Owing to the low Reynolds number [39] in the microfluidic channel, laminar flow of the inflowing stimulant and buffer solutions was established during the “off” state of the transducer, as shown in Figure 2-3 (a). Once the transducer was turned “on”, all bubbles trapped within the horseshoe structures were excited simultaneously. The streaming and sideward mixing of the liquids at the trapped bubbles in a stepwise fashion resulted in a gradient of the stimulant. Figure 2-3 (b) shows the gradient generated around the horseshoe structures and the region far away from the bubbles. We adjusted the voltage such that a mixing ratio of 1:1 was achieved between subsequent bubbles, which ensured an exponential decay chemical profile. Fluorescence distribution across the channel at positions 1–6 (after passing each bubble, indicated in Figure 2-3 (b)) is shown in Figure 2-3 (e). We observed a step-like intensity function at position 1, due to absence of mixing between the Dextran-FITC and PBS solution. At position 2, Dextran-FITC and PBS solutions were mixed in the region between the front end of the first and the rear end of the second horseshoe structure. Similarly, the subsequent oscillating bubble progressively mixed and diluted the Dextran-FITC until an exponential decay gradient profile was established at position 6, as depicted in Figure 2-3 (e) (yellow line). As the stimulant approaches the rear end of the channel, diffusion-induced mixing of the stimulant and buffer solutions results in smoothening of the generated chemical gradient, as shown in Figure 2-3 (f) (V_{pp} = 12 V). The measured intensity profiles fit well to a first-order exponential decay function, confirming that a 1:1 mixing ratio of the subsequent bubbles ensures an exponential gradient profile.
2.3 Conclusion

In summary, we have demonstrated standing surface acoustic wave-based acoustic tweezers that can manipulate single particles/cells/organisms in a microfluidic chip. This acoustic device has significant advantages in biocompatibility and versatility. The lower power density requirement renders our technique extremely biocompatible. The simple structure/setup of these acoustic tweezers can be integrated with a small RF power supply and basic electronics to function as a fully integrated, portable, and inexpensive particle-manipulation system. The technique’s versatility has three aspects: 1) it is capable of manipulating most microparticles regardless of shape, electrical, magnetic, or optical properties; 2) it is capable of manipulating objects with a variety of length scales, from nm (if we use higher SAW frequency) to mm (as demonstrated in
C. elegans); and 3) it is capable of manipulating a single particle or groups of particles (e.g., tens of thousands of particles). The acoustic tweezers’ versatility, biocompatibility, and dexterity render them an excellent platform for a wide range of applications in the biological, chemical, and physical sciences including the fundamental studies of mechanical properties of micro and nano scale particles such as cells, DNAs, proteins, and molecules. Additionally, the ability to massively move particles with great speed (up to 1600 μm/s) could facilitate this technique a key tool in many high-throughput assays such as cell sorting and separation. Finally, this device could be used to help researchers examine the behavioral and neuronal response of small organisms (such as C. elegans) to mechanical and chemical stimuli
References


Chapter 3

Programmable chemical waveform generation and switching using acoustically activated bubbles

3.1 Introduction

Cellular response to chemical microenvironments varies depending on the spatiotemporal characteristics of stimulus signals, which are central to many biological processes including gene expression, cell migration, differentiation, apoptosis, and intercellular signaling. Precisely mimicking the dynamics of the local chemical concentration found in vivo can assist in the elucidation of the underlying molecular mechanisms and can enable complex tasks in vitro such as tissue engineering. To date, research in this field has been limited to digital chemical waveforms with little control over the temporal nature of the signal. In this chapter, I will discuss a novel acoustically activated, bubble-based microfluidic system for generating arbitrary temporal chemical waveforms (both digital and analog), by mixing the stimuli and buffer solutions in a time-dependent fashion. Our approach permits continuous modulation of the signal characteristics including shape, frequency, amplitude, and duty cycle, with frequencies reaching up to 30 Hz. Furthermore, through integration of multiple bubbles in a single microchannel, we demonstrate fast switching between multiple distinct stimuli, wherein the waveform of each stimulus can be independently controlled. We demonstrate the applicability of our device for studying fast biomolecular processes in cells by showing that the activation and internalization of G-protein coupled receptors (GPCRs) requires that the chemical
stimulus, epinephrine, is present for at least 100 ms. With its advantages in functionality and versatility, the chemical waveform generation and switching method presented herein is expected to become a powerful tool in understanding the dynamics of various biomolecular processes.

3.2 Motivation

Studies have shown that signals with identical chemical composition can result in different biological outcomes [1–9] when given with different spatio-temporal characteristics. Generating these environments with high spatial and temporal fidelity will provide researchers with important degrees of freedom when studying dynamic biological processes [10–12]. A seminal work was carried out by Dolmetsch et al. where they exposed T cells with varying frequency of liquid containing calcium to study the frequency response of gene expression [8]. Their macrofluidic setup was used, as shown in Figure 3-1 (left), where one reservoir contained the calcium ions and the other contained EGTA. The frequency of the liquid containing the calcium was controlled by a PC-controlled valve. The study had shown that the frequency and the amplitude of the chemical stimulations lead to specificity of gene expression. Figure 3-1 (a) (right) shows that when step stimuli was exposed to T cells; NFAT, Oct/OAP and NFκB genes were simultaneously expressed and the expression level increased with the increasing stimuli concentrations. On the other hand, while maintaining the concentration constant and by varying the frequency of stimulation, the level of these genes expressed differently. For example, as the stimuli frequency was increased, NFAT and Oct/OAP genes were not
turned on, while gene NFκB was expressed, shown in Figure 3-1 (b) (right). Their findings proved that gene expression is encoded not only in the concentration (or amplitude) of the stimuli but also in the frequency of the stimuli. Though important but still a lot needs to be explored [8].

Figure 3-1 Right (a)–(c) Schematic of macrolevel waveform generation. Left (a)-(b) Gene expression level varies over oscillating stimulation [8].

As of yet, the temporal aspects of gene expression is little studied. How a particular gene behaves under different signal characteristics including shape, frequency, amplitude, and duty cycle is extremely important from the fundamental studies point of view (Figure 3-2). Most studies that exist in the literature are goal specific [2], [8]. Thus, there is a need for a versatile chemical waveform to study gene expression under different chemical signal characteristic and with varying concentration.

Microfluidic technology is usually preferred over macrolevel liquid manipulation because it offers high-throughput experimental conditions, less wastage of expensive human reagents and has the potential to miniaturization and automatic operation. However, a fundamental challenge in microfluidics, attributed to low Reynolds number associated with its dimensions, is to precisely control the temporal aspect of the stimuli.
Figure 3-2 A gene can behave differently under varying chemical input stimuli.

Although externally actuated valves [13] and recently passively controlled microfluidic circuit [14] has been utilized to control the liquid flow oscillation, it is limited to significant off-chip hardware (vacuum pumps, switch solenoids, etc.[15]) and moreover they don’t allow modulation of chemical concentration. On the other hand, chemical dilution, a high demand operation and widely used as assay in chemical and biological laboratories has proven to be extremely difficult to implement in microfluidic settings [16]. The existing concentration modulation methods are limited to slow modulation rate. Both switching and chemical concentration modulation concepts are difficult to implement but are extremely important functions to study the cell behaviour.
3.3 Brief Literature Review

Microfluidics has recently been utilized towards generating chemical stimulation [10], [17–19] because it offers a level of fluidic control [20] often lacking in bulk systems. While remarkable progress has been made toward the spatial modulation of chemical stimuli in microfluidics, e.g., generation of chemical gradients [10], [21–23], temporal modulation has received limited attention. Kuczenski et al. demonstrated and developed an external pressure control method to change the interface to stimulate the cells with liquid containing ionomycin to study the release of calcium ions, as shown in Figure 3-3 [24]. Hersen et al. developed a system similar to Kuczenski et al., where they maneuvered the stimuli interface by a 3- ways electro valve in a time-dependent fashion to study the HOG MAP kinase pathway of yeast cell under varying frequency [17], their concept is shown in Figure 3-4. Leslie et al. used an elegant concept utilizing fluidic diodes and capacitor in combination to create microfluidic flow oscillator [14]. The architecture of their system is shown in Figure 3-5 (a). The chip contains input reservoirs, pairs of capacitors, diodes, resistors and was connected to an oscillatory pressure unit, Figure 3-5 (b). Operation of the device was demonstrated by oscillation between red and blue food dyes at different frequencies, which are characterized in Figure 3-5 (c)-(d) [14].

Techniques for temporal manipulation of chemical stimuli [14], [25–31] are usually based on the concept of switching between two or more liquid inlets [14], [15], [17], [26], [30], [31], analogous to a multiplexer in electronics. These designs generally require sophisticated fabrication methods [13–15], [30], [31], exotic materials [25], [32], [33] and/or have working frequencies limited to 1 Hz [15]. Moreover, although the existing
techniques generate digital waveforms conveniently, most of them cannot achieve continuous modulation of the amplitude and/or frequency (i.e., analog waveforms). Furthermore they are limited to numerous bulky off-chip devices that will potentially be difficult into developing into a versatile and a high throughput system [15].

Figure 3-3 (a) Chemical stimulations by shift in interface utilized by pressure control feedback. (b)-(c) Demonstrates the shift of interface by ink [15].
Sinclair et al. developed an open volume microfluidic chemical generator that was mounted on a programmable scanning stage (PSS) [26], [34]. The microfluidic chamber was maneuvered by controlling the PSS, as a result when liquids of different chemicals are injected into the multiple inputs of the microfluidic chamber; it created a collimated stream of liquids flow [34], as shown in Figure 3-6. Due to liquid injecting at flow rate of 3 mm/s, the liquids stream remained laminar. Consequently a cell was attached via patch
clamp method and brought close to the liquid stream and the stage was maneuvered in the y-direction, as a result the stationary cell was exposed to different chemicals [34].

Figure 3-6 (a) Microfluidic device utilized to generate binary chemical waveforms. The microfluidic chip is mounted and controlled by a programmable scanning stage, such that when a stationary cell was brought close to the chamber with liquid flow, the cell experience different chemical stimuli. (b) Optical micrograph of the microfluidic chamber. (c) Scanning electron microscopic image of channels. (d) Image showing the collimated fluorescence stream from the 32 channels [34].

Though their approach was innovative but the method is not conducive to multiple cells studies. The flow rate (3mm/s) required maintaining the stream laminar was high. At 3 mm/s, the flow may damage the cell by shear rate or unexpectedly turn on shear-induced genes. Moreover, since the system is open volume, a slight perurbation will damage the flow profile.
Kress et al. demonstrated an optical method to manipulate the chemical environment [33]. The microsource was loaded with fMLP chemoattractant and is maneuvered by an optical tweezer. By controlling the motion of the microsource, they can entice the neutrophil to follow and perform migration. Figure 3-7 (a)-(f) shows the neutrophil polarization and migration towards the chemoattractant filled microsource. Figure 3-7 (g) shows the deformation of the neutrophil contour during the migration process. Figure 3-7 (i) shows the gradient direction generated by the microsource and the corresponding cell orientation over time. Though this is an interesting method to generate chemical stimuli over time, one can study couple of cells at a time, making this system not susceptible to high throughput studies.

Figure 3-7 (a-f) Chemically loaded bead was optically manipulated to maneuver the neutrophil. (g) Cell contours demonstrating cell deformation during directed migration towards the bead. (i) Direction of the chemical gradient and the corresponding cell orientation versus time [33].
Controlled dilution of chemical is an operation that is widely used as assay in chemical and biological laboratories [16]. Though dilution is a basic task, it is extremely challenging in microfluidics settings [16]. Niu et al. demonstrated a microdroplet method to dilute sample [16]. Their design consists of an input channel, the dilution chamber, pillars structures and an output channel, as shown in Figure 3-8 (a). A mother droplet which contains the food dye is trapped inside the dilution chamber. Then a buffer droplet was passed through the input channel and it transiently got trapped in the pillar structures. Then the mother droplet transfer food dye into the trapped droplet and eventually the incoming droplet got released, the process is show in Figure 3-8 (b). They have shown that the concentration of food dyes in each droplet decreases exponentially by the droplet number $n$ [16]. Sequence of droplets flowing through the output channel with different concentration was shown in Figure 3-8 (c). Though this is a nice method to dilute the chemical, it’s not flexible to alter the concentrations, and usually these kind of devices need to be connected with other setups to carry out any useful biological or chemical functionalities.

### 3.4 Results

Here I have demonstrated that an oscillating bubble in an acoustic field provides a unique and versatile method to generate arbitrary temporal chemical waveforms by mixing stimulus and buffer solutions in a time-dependent manner. Our approach is capable of generating not only digital chemical waveforms, but also analog waveforms whose characteristics, including shape, frequency, amplitude, and duty cycle, can be

Figure 3-8 (a) Schematic of the dilution module. (b) Images sequence showing the process of dilution. (c) Sequence of droplets flowing through the output channel with different concentration [16].
conveniently modulated. Furthermore, by trapping multiple bubbles into a single microchannel, we demonstrate switching between two distinct stimuli, wherein the waveform of each stimulus can be independently controlled. Trapping multiple bubbles on a single chip will also enable us to integrate the chemical waveform generator and switch presented here with other bubble-based, on-chip functions such as cell/particle manipulation [35–37], mixing [38], [39], separation/sorting[40], [41], and pumping [42] thus reducing dependencies on off-chip devices. To demonstrate the capability of our device to characterize fast biological processes, we show that the temporal response of epinephrine-induced G-protein coupled receptor (GPCR) activation and its subsequent internalization in cells can be monitored using our microfluidic device by precisely controlling the duration of stimulation.

**Chemical waveform generator using acoustically activated bubbles.**

Figure 3-9 (a) shows the schematic diagram of our microfluidic chemical waveform generator. A horse-shoe structure (HSS) inside a polydimethylsiloxane (PDMS) microfluidic channel uses surface tension to trap and support a single bubble; the structure also helps determine the size of the bubble. When driven by an adjacent piezoelectric transducer, the membrane of the trapped bubble oscillates. Like the vibration of strings or the oscillations of a spring-mass system, each bubble has a size-dependent resonance frequency which results in maximum oscillation amplitude. At this frequency, frictional forces develop at the interface of the bubble and the surrounding medium, giving rise to a pressure gradient in the fluid that results in the prominent
recirculating flow regions depicted in Fig. 3-9 (b). This phenomenon is commonly referred to as acoustic “microstreaming” [35], [43].

Figure 3-9 Concept of waveform generation. (a) Schematic of the experimental setup (see also Supporting Information on experimental details). The piezoelectric transducer, which generates low-intensity acoustic waves, is placed adjacent to the microfluidic channel on a glass slide. The acoustic waves drive the bubble trapped in the horse-shoe structure (HSS), which is placed at the interface of the co-flowing liquids. (b) Experimental observation of acoustic microstreaming and flow recirculation during the bubble oscillation. (c–i) The mixing of red and blue dyes is captured with high-speed imaging. The region of interest (ROI) for the output waveform was chosen ~ 300 μm downstream of the HSS (i.e., past the recirculation zone), in the bottom half of the channel. The chemical waveforms were determined by the optical density of the ROI when ink and buffer solutions were used.
When the trapped bubble is excited, the counter-rotating vortices developed during microstreaming disrupt the clean liquid-liquid interfaces which are characteristic of the laminar flow regime in the microchannel. The vortices drastically enhance the mass transport along the direction perpendicular to the flow, effectively mixing the inlet solutions (On state). We observed the mixing process through fast imaging (1200 frames/s), shown in Figure 3-9 (c)-(i). Complete mixing of the fluids occurred in less than 30 ms. When the excitation was removed, the mixing stopped and the characteristic laminar flow returned (Off state). The fast responses of the electric and acoustic systems allowed the device to directly convert electrical signals into chemical waveforms—effectively implementing all the capacities of the function generator.

**Generation of digital chemical waveforms.**

To demonstrate the device’s functionality, we generated a variety of different chemical waveforms, as shown in Figure 3-10 (b)-(e) using the function generator to control the acoustic excitation. In the experiments, we infused the two inlets with dye and a buffer solution at identical flow rates (6 µl/min). The optical density of a specified region of interest (ROI) was used to determine the mixing efficiency and give a rough estimate of the stimulant concentration. A square waveform was generated by simply switching the transducer on and off. The frequency (Figure 3-10(b)) and duty cycle (Figure 3-10(d)) of the signals were controlled by appropriately timing the on and off states of the bubble oscillation. Burst/pulse signals were generated (Figure 3-10 (c)) by lowering the transducer excitation duration to less than the complete mixing time (30 ms). We also
show that the frequency of the chemical signal can be modulated without interruption (Figure 3-10 (e)), with the potential for a continuous frequency sweep.

Figure 3-10 (a) Generation of various chemical waveforms in the region of interest (ROI) marked green liquid region. (b) Square wave. (c) Duty cycle. (d) Burst mode. (e) Tunable frequency.

**Generation of analog chemical waveforms.**

In Figure 3-10 (b-e), we showed several chemical waveforms with constant maximum and minimum amplitudes, *i.e.*, digital waveforms. To generate analog signals, such as sinusoidal or triangular waveforms, it is essential to dynamically vary the amplitude of the stimulus (*i.e.*, the concentration of the stimulus). This amplitude modulation was attained by continuously mixing the stimulus and buffer solutions while changing the relative flow rates of the inlets. As the relative flow rates change between two fluids in a
Figure 3-11 (a) Image sequence showing the shift of interface by altering the flow rate. (b) Graph of flow rate utilized in achieving amplitude modulation. (c) Sine wave.

In the microchannel, the location of their interface shifts along the width of the channel due to the difference in inlet pressures, Figure 3-11(a). This controllable interface can be used to vary the proportion of each fluid that is mixed by the bubble, resulting in a tunable output concentration of the stimulus.

The applied flow rate pattern and the respective chemical waveform are shown in Figure 3-11 (b) and Figure 3-11(c), respectively. We note, however, that the amplitude modulation frequency will be limited by the response of the flow pump. This concept can be readily used for applications such as single-shot chemical kinetics studies [44], [45].
**Fast amplitude (concentration) modulation.**

We note, however, that the amplitude modulation frequency is limited by the slow response (approximately second time-scale) of the flow pump. To achieve rapid amplitude modulation, independent of the pump response, we designed a three-inlet channel with a HSS at the center. The stimulus (ink) was injected in the center at a fixed flow rate of 1 µl/min, while the buffers (water) were infused through the other inlets at a fixed flow rate of 2.5 µl/min, as shown in Figure 3-12 (a). The trapped bubble was excited close to its resonance frequency. The amplitude of oscillation, which scales linearly with the applied voltage, is tuned by the function generator. As the voltage is increased, the mixing distance, $d$, of ink and water increased, in a linear fashion. Figure 3-12 (b) shows the step decrease in the stimulant concentration for a step increase of the voltage applied to the transducer in the selected ROI marked in Figure 3-12 (a). Note that the voltage can be tuned using smaller steps to achieve finer amplitude modulation. The ink concentration decreases because more buffer solution is mixed for a fixed volume of the stimulant. The change in concentration was attained almost instantaneously (Figure 3-12 (b) inset), which allowed us to tune the desired concentration.

**High-frequency characterization.**

The digital frequency response of our device is intrinsically limited by the mixing capabilities of the bubble, properties of the fluids (e.g., density and surface tension), flow velocity, and location of the ROI. To quantify the high-frequency response of the device, we compared the photointensity of the ROI during partial mixing (pulse width less than the total mixing time) to the intensity at complete mixing to obtain a quantitative measure
Figure 3-12 Rapid amplitude (concentration) modulation. (a) Schematic of the experimental setup for rapid amplitude modulation. The channel consists of a single HSS with three inlets and one outlet: inlets 1 and 3 were infused with a buffer solution (water), and inlet 2 was infused with stimuli (ink). When the bubble was acoustically activated, ink mixes with water with a mixing distance, $d$. (b) Graph shows dilution of the ink for increasing voltage in the selected ROI marked in a. The inset shows the raw amplitude modulation data, suggesting rapid modulation (less than 100 milliseconds).

of the total mixing efficiency. Figure 3-13 (a) shows the relative intensity, indicative of mixing efficiency, at increasing pulse width duration for four different flow rates; the observed response is typical of a low-pass filter, where low frequencies show distinct
Figure 3-13 (a) Characterization of frequency response. Frequency response of waveform generation with flow rates of 3, 5, 7, and 11 µl/min. Error bars represent the standard deviation from a minimum of five measurements. (b-d) Waveform generation utilizing 17 ms pulse duration (marked as dotted circle) at 11 µl/min flow rate. (b) 16 Hz for 60 ms trigger interval. (c) 33 Hz for 30 ms trigger interval. (d) at 20 ms trigger interval.

chemical signals, but higher frequencies blur into a continuum. As seen in the inset of Figure 3-13 (b-d), the demixing time (i.e., the falling time), which is dependent on the flow rate, is the rate-limiting factor in the device’s frequency response. Despite partial mixing, distinct chemical pulses can be generated at frequencies greater than 30 Hz—more than an order of magnitude faster than previous microfluidic designs (~1 Hz) [15].

**Chemical switching using multiple bubbles.**

While the generation of single chemical waveform is vital to a variety of biochemical studies, dynamically switching between or concurrently applying different chemical stimuli is also important when studying more complex dynamic systems, such as cell
signaling pathways [7], [9] or cascades of biochemical reactions [46], [47]. In principle, these studies require logic-type control utilizing multiple waveform generators. Independently mixing multiple waveforms within our microchannel requires multiple trapped bubbles with different resonance frequencies so they may be excited separately. The resonance frequency of a bubble is governed by its geometry (i.e., radius) and the properties of the liquid. Assuming a constant liquid medium, we used HSS geometry to effectively alter the fundamental resonance frequency of the bubbles. Preventing cross-excitation due to higher-order harmonic modes of oscillation was the main challenge. We pre-screened nine HSS geometries that varied in width (Figure 3-14 (a)). To experimentally determine the resonance frequency of each bubble, we swept the excitation frequency from 10 kHz to 60 kHz in 100 Hz increments while visually monitoring the oscillation amplitude for a distinct peak. The results are shown in Figure 3-14 (b).

![Image](image.png)

Figure 3-14 Characterization of bubbles’ resonance frequencies. (a) A 3 x 3 array of HSSs with different dimensions used to obtain different sized bubbles. (b) Graph of both the experimental (green triangles) and theoretical (red circle) results for resonance frequencies of different-sized bubbles. Error bars represent the standard deviation from three measurements conducted from three different microfluidic chips with depths of 65 µm.
Figure 3-15 (a) shows a schematic diagram of the device used for switching between two different chemical signals. The channel has three inlets and one outlet; inlets 1 and 3 (peripheral regions) were infused with different chemical signals (red and blue dyes for demonstration), and inlet 2 pumped a buffer solution (water) into the central region that served as our ROI. Distinct HSSs (60 x 90 µm and 110 x 165 µm) were positioned at each liquid-liquid interface; the corresponding bubbles had resonant frequencies of 29.5 kHz and 14.7 kHz (see Figure 3-15 (a)). Cross-excitation of the bubbles at the above frequencies was negligible, shown by the microstreaming bead test captured in Figure 3-15 (b). Figure 3-15 (c) lays out the binary chemical circuitry: when bubble A was activated at $f = 14.7$ kHz, only the red dye mixed with the water to fill the region of interest (Figure 3-15 (d), bottom panel). Conversely, when bubble B was activated at $f = 29.5$ kHz, only the blue dye mixed with the water (Figure 3-15 (d), top panel). Switching between the red and blue dyes was achieved by alternating between the two excitation frequencies (Figure 3-15 (e)). The direct conversion of electrical signals into chemical waveforms allows this device to access all of the previously demonstrated functions of the waveform generator, including frequency and amplitude modulation.

**GPCR activation and internalization.**

To maintain homeostasis in multicellular organisms, cells must be able to both sense their external environment and communicate intracellularly via chemical mediators. G protein-coupled Receptors (GPCRs) play a significant role in these processes by binding external
Figure 3-15 Bubble-based switching between multiple stimuli. (a) Schematic of the experimental setup for chemical switching. The microfluidic channel contains HSSs of different sizes, and subsequently bubbles of different sizes that are independently driven by transducers bonded to the substrate adjacent to the channel. (b) Top, visualization of microstreaming from the bubble trapped in HSS A (red), while no streaming is observed in the bubble trapped in HSS B (blue) at an excitation frequency of 14.7 kHz. Bottom, visualization of the microstreaming from the bubble trapped in HSS B while no streaming occurs in HSS A at an excitation frequency of 29.5 kHz. (c) Table showing the concept of binary logic circuitry. (d) Results demonstrating switching between the blue and red dyes (see Supporting Video 6 for further illustration). (e) Graph of experimental data for switching between red and blue dyes in the selected ROI marked in (d).

stimuli and initiating transduction pathways that lead to cellular responses. Upon prolonged or repeated exposure to agonists, GPCRs may become desensitized and internalized in clathrin-coated pits [48–51]. We monitored this process using a stably transfected cell line expressing GFP tagged β₂-AR. The microfluidic experimental setup is shown in Figure. 3-16 (a), wherein a single trapped bubble is used to generate a temporally controlled pulse of chemical stimulant, epinephrine (5 µM), over a culture of human embryonic kidney (HEK) cells. Without treatment, the GFP fluorescence is
predominantly located at the cell plasma membrane (Figure 3-16 (b) and Figure 3-17 (a)-(b)). Upon continuous treatment with epinephrine for 45 minutes, GFP tagged β2-ARs are translocated from the plasma membrane surface to the cell interior, as indicated by the decreased fluorescence at the cell boundary and the increase in β2-AR-loaded endosomes (Figure 3-16 (b) and Figure 3-18 (a)-(c)). A punctate staining pattern within the cytoplasm, arising from endosomes containing the GFP tagged β2-AR, was used as the means to infer whether the applied pulse of epinephrine led to β2-AR activation and internalization. When the stimulant pulse was applied for a duration of 100 ms, no significant internalization of β2-ARs was observed, as shown in the time series of Figure 3-16 (c). Even after 45 minutes, the fluorescently-labeled β2-ARs are largely observed at the membrane boundary with no significant increase in labeled endosomes in the cell interior. Conversely, when the stimulant pulse was applied for a duration of 5 seconds, significant internalization of β2-ARs takes place (Figure 3-16 (d)) with an onset time of 15 minutes. This result is significant in that the agonist stimulation duration as short as 5 sec is sufficient to cause receptor internalization. Epinephrine is an endogenous agonist for the β2-AR with rapid on and off rates, and its residence time (reciprocal to the off rate) to the β2-AR is estimated to be less 0.2 sec [52]. However, the agonist-stimulated β2-AR internalization in HEK cells is dependent on phosphorylation of the agonist-stimulated receptor by G-protein-coupled receptor kinase 2 (GRK2) followed by binding of β-arrestins to the phosphorylated [53], [54] and requires persistent agonist occupancy [54], [55]. The GRK-dependent phosphorylation is the rate-limiting step in the receptor internalization and often takes several minutes for completion [54], [56]. Our result
clearly demonstrates the temporal nature of β2-AR activation and internalization due to epinephrine binding in cells, a result which would be difficult to demonstrate in cells using conventional techniques (e.g. single-molecule fluorescence spectroscopy). Given that most signaling events take place with different spatial-temporal dynamics, our microfluidic system offers a promising tool for controlling and characterizing the dynamics of biomolecular processes in the native cellular context.

Figure 3-16 Temporal response of GPCR internalization using chemical waveform. (a) Setup of chemical switching. A human embryonic kidney (HEK) 293 cell line which was stably transfected with a GFP-labeled GPCR was cultured inside the microfluidic waveform chip. The stimulant (epinephrine, 10 µM) and buffer (culture medium) are injected at 1 µl/min into the channel. (b) GPCR internalization upon stimulation by 5 µM epinephrine. Image taken at 45 min demonstrating internalization (note experiments were performed in Petri dishes). (c) No internalization of the labeled GPCR was observed in the microfluidic chip at a pulse width of 100 milliseconds stimulant (red arrow indicates the fluorescently-labeled GPCRs remain unchanged at
the membrane boundary). (d) Internalization of the labeled GPCR was observed in the microfluidic chip at a pulse width of 5 seconds of epinephrine (yellow arrow indicates internalization with an onset time of 15 minutes).

Figure 3-17 A human embryonic kidney (HEK) 293 cell line was stably transfected with a GFP-labeled GPCR. HEK cells were cultured on the petri dish and no internalization of the labeled GPCR was observed in the absence of any stimuli. Confocal images showing (a) top view and (b) side view.

Figure 3-18 GFP tagged β2-ARs are translocated from the plasma membrane surface to the cell interior indicated by punctate staining pattern within the cytoplasm in the presence of epinephrine stimulation. Confocal images showing (a) top view, (b) side view, and (c) three-dimensional view.
3.5 Conclusion

Chemical waveforms, when compared to constant chemical signals, can have markedly different effects on cellular signaling pathways which receive, transmit, process, and implement directions from chemical stimuli. With the on-chip waveform generator and switch reported here, it is possible to measure the dynamics of receptor-mediated signaling and other cellular responses to small molecules. The device can also be used to study cellular processes that span a wide range of time scales, from milliseconds (56–58) to hours. Finally, generating waveforms in continuous flow also eliminates the abrupt changes in shear stress at the cell membrane in segmented flow devices, more closely mimicking the *in-vivo* chemical signals. These precisely controlled chemical waveforms could play a significant role in measuring the kinetics of fast cellular signaling events, explaining the specificity and efficiency of gene expression, and in the development and validation of time-released drugs.

3.6 Methods

**Device fabrications.**

A single-layer polydimethylsiloxane (PDMS) microchannel was fabricated using the soft lithography and the mold replica technique. In short, a silicon mold for the microchannel was patterned in photoresist (Shipley 1827, MicroChem, Newton, MA) and etched with Deep Reactive Ion Etching (DRIE, Adixen, Hingham, MA). The mold was then coated with 1H,1H,2H,2H-perfluoroctyl-trichlorosilane (Sigma Aldrich, St. Louis, MO) to
reduce its surface energy and any subsequent damage to the PDMS channel during the demoulding process. Sylgard™ 184 Silicone Elastomer Base and Sylgard™ 184 Silicone Elastomer Curing Agent (Dow Corning, Midland, MI) were mixed at a 10:1 weight ratio and cast onto the silicon mould. The uncured PDMS on the silicon mold was then degassed in a vacuum chamber for 2 h to remove any air bubbles and later cured at 65 °C for 45 min. After removing the cured PDMS from the mold, the inlets and the outlets were drilled into the PDMS using a silicon carbide drill bit (model 220/395, Dremel). The microfluidic channel was then bonded to a micro cover glass, which had been pre-treated with oxygen plasma. A piezoelectric transducer (model no. 273-073, RadioShack) was then attached to the glass slide adjacent to the channel using epoxy (Permatex 84101).

**Experimental details.**

The glass slide, including the microfluidic channel and the piezoelectric transducer, was mounted on a Nikon TE-2000U optical microscope stage. Ink (PAR3001100, Parker) or food dye (Assorted/ NEON, McCormick) was infused into the channel through a 1 ml syringe (Becton Dickinson) by automated syringe pumps (KDS Legato 210, KD scientific, Holliston, MA, USA). Once the bubbles were stably trapped with a smooth flow, the transducer was connected to a function generator to control the bubble activation/deactivation via a function generator (Hp8116A /Tektronix AFG 3011). The driving voltages used in the experiments were 8‒16 VPP.

**Data/image acquisition and analysis.**
Data acquisition for the waveform generation in Figure 3-10 was directly achieved by ROI selection during the experiment using InVivo (MediaCybernatics) microscopy software, connected to a CoolSnap HQ2 (Photometrics) CCD camera. The images in Fig. 3 were captured at 1200 fps (to record the fast dynamics of each stimulus) and later processed through a Matlab code. The rest of the images were captured by Nikon D3S or CoolSnap CCD cameras. All the videos were captured by Nikon D3S or Casio EX-F1. Raw movie files were encoded into stack of images, and further processed using Image J software. (http://rsb.info.nih.gov/ij/)

**Design and dimensions of microchannels**

Figure 3-19 shows two images of the horse-shoe structure (HSS). Figure 3-20 shows the 3 x 3 array of HSS used to prescreen the appropriate dimensions of the two HSSs that were later utilized in the chemical switching device. Figure 3-21 shows the design and dimensions of microchannels used for (a) chemical waveform generation and (b) switching.

![Figure 3-19](image_url) (a) An SEM image of the HSS within the PDMS microfluidic channel. (b) Optical image showing the top view of the HSS structure.
Figure 3-20 The 3 x 3 array used to pre-screen the HSSs resonance frequencies to ensure no cross-excitation in the chemical switching experiment.

Figure 3-21 Design and dimensions of the microchannels used for (a) chemical waveform generation and (b) switching.

**Optimized flow rate for bubble trap**

The outlet flow rates were set to optimize for repeatable bubble traps within the HSS (listed in Table 1).
<table>
<thead>
<tr>
<th>Device</th>
<th>Flow rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 bubble</td>
<td>6 µl/min</td>
</tr>
<tr>
<td>3 x 3 array of HSS</td>
<td>12 µl/min</td>
</tr>
<tr>
<td>2 bubble</td>
<td>12 µl/min</td>
</tr>
</tbody>
</table>

Table 3-1 Optimized flow rate for repeatable bubble traps within the HSS.

**Input signals given to the piezoelectric transducer**

Figure 3-22 shows the electrical signals applied to the piezoelectric transducer by the function generator driving at the resonance frequency of the bubble.
Figure 3-22 Input signal given to the piezoelectric transducer by the electrical function generator, driving at the resonance frequency of the bubble (see inset) for (a) Square wave generation. (b) Burst cycle generation. (c–e) 20, 50, 80% duty cycle.
**Impedance and phase angle measurements of the piezo transducer**

The resonance frequency of each bubble is independent of the transducer’s response. We tested this by measuring the resonance frequency of the transducer attached to the glass cover slip (Figure 3-23). The transducer’s resonances are indicated by sharp peaks in the phase angle plot. The bubble resonances in the experiments were observed between 15 kHz and 45 kHz, and no significant transducer resonances were observed in that range of frequencies. This data indicates that the bubble resonances we observed in these experiments are independent of the transducer resonances and solely dependent on the geometry of the bubble.

![Figure 3-23](image)

Figure 3-23 Impedance and phase shift measurements of the piezoelectric transducer. (a) Impedance measurements of the transducer attached to the glass cover slip. (b) Phase shift measurements of the piezoelectric transducer attached to the glass cover slip. The resonances of the transducer are indicated by sharp peaks in the phase angle plot.

**Dynamic mass redistribution (DMR) assay**

Label-free DMR assays enabled by RWG biosensor were performed using Corning® Epic® BT system, a swept wavelength interrogation system that is capable of imaging whole RWG biosensor microplates with a spatial resolution of 90 µm [57]. This system uses a light beam from a tunable light source to illuminate whole 384-well biosensor
microplate, and a high speed complementary metal-oxide semiconductor (CMOS) digital camera to record the escaped and reflected resonant lights from the whole plate. The tunable light source sweeps the wavelength range from 825 to 840 nm in a stepwise fashion to identify the resonant wavelength at each location. Total 150 spectral images were acquired within a single sweeping cycle (3 sec), and were then processed into sensor resonant wavelength or DMR image in real time.

For receptor signaling, cells were first harvested from T75 flask using trypsin/ethylenediamine-tetraacetic acid, and re-suspended in the completed medium. Cells were seeded onto the fibronectin coated biosensor microplates using a seeding density of 12,000 cells per well in 40 μl the complete medium, and cultured at 37°C with 5% CO₂ inside a cell culture incubator for about 22hrs to reach a confluency of ~95%. Afterwards, the cells were washed three times using a plate washer (Bio-Tek Microplate Washers ELx405™, Bio-Tek, Winooski, VT), and maintained in the assay buffer (HBSS) for about one hour to reach equilibrium at 26°C. After establishing a 2min baseline, DMR assays were then initiated by adding the HBSS buffered compound solution and the compound-induced DMR was recorded in real time.

Results showed that epinephrine gave rise to a single EC₅₀ of 11.4±1.2 nM (n = 4) in the parental cells (Figure 3-24). In contrast, in the engineered cells epinephrine displayed an assay time dependent potency; that is, the potency to reach the early peak response was found to be biphasic with two well-separated EC₅₀ (0.58±0.04 nM, and 69.5±7.1 nM, respectively), while the potency to reach the late plateau response was
monophasic with an EC$_{50}$ of 124±11 nM. Nonetheless, these results suggest that when overexpressed in HEK293 cells the β$_2$AR-GFP is functional.

![Figure 3-24. DMR characterization of the β$_2$-ARs. (A, b) The real-time DMR signals of epinephrine at different doses in (a) the parental HEK293 cells; (b) the HEK-β$_2$AR-GFP cells; (c) the dose-dependent DMR amplitudes of epinephrine in both cell lines. For the parental HEK cell line which endogenously expresses the β$_2$-ARs at low level, the DMR amplitude at 50 min poststimulation was plotted. For the HEK-β$_2$AR-GFP cells the DMR amplitudes at both 5 min and 50 min poststimulation were analyzed. For (a-c), the data represents mean ± s.d. (n =4).](image-url)

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VITA
Daniel Ahmed

Education
Ph.D., Engineering Science and Mechanics, The Pennsylvania State University, USA (2013)
M.S., Engineering Science and Mechanics, The Pennsylvania State University, USA (2009)

Honors and awards
Breneman Chair University Scholarship, The Pennsylvania State University (2013)
First place, Penn State ESM Today Graduate Research Symposium (2013)
First place, Penn State ESM Today Graduate Research Symposium (2012)
CHEMINAS Young Researcher Award at MicroTAS (2011)
Grand prize, Penn State ESM Today Graduate Research Symposium (2009)

Selected journal publications


8. Rotation of single cell and small living organisms, Daniel Ahmed, Mengqian Lu, Jarrod B. French, Stephen Benkovic and Tony Jun Huang (to be submitted to Lab on Chip).