Effect of Temperature and Iron-Oxide Nano-particle inclusions on the Ultrasound Vaporization Pressure of Perfluorocarbon Droplets for Disease Detection and Therapy

A Thesis submitted in partial satisfaction of the Requirements for the degree Master of Science in Bioengineering

by

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2009
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Chair

University of California, San Diego
2009
Dedication

I would like to dedicate this thesis to my mom, dad and sister who were always there for me and have given me countless bits of encouragement, support and prayers.

Thank you
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Introduction: The longer circulating time and larger particle count of perfluorocarbon (PFC) droplets make them more effective as targeted contrast agents compared to microbubbles. It has also been shown that conversion of PFC droplets to microbubbles improves ultrasound (US) sensitivity to emulsions by 10 orders of magnitude. PFC droplets, with their higher boiling point and smaller particle size are more desirable to prevent spontaneous vaporization and maximum accumulation of emulsions. However, the high US energy required to induce phase conversion in these emulsions is not clinically feasible. We hypothesized that by increasing the temperature and using Iron-oxide nano-particles (IONP) as nucleation sites within the more stable and submicron
droplets, the US energy required to vaporize them may be lowered. **Material and method:** A sample of 60%w/v iron-oxide loaded PFC droplets with mean diameter of 200nm was manufactured and characterized. A phantom was designed to allow for the interaction between the US energy and droplets. A high intensity focused ultrasound system (HIFU) was used to generate US pressures and a heat exchanger pump was used to control the temperature while emulsions of PFHB and PFP with or without IONP were circulated through the chamber. A harmonic imaging system was also used to detect the generated microbubbles. **Discussion and result:** The effect of temperature and IONP on the vaporization rate and threshold of PFC emulsions were determined. It was shown that presence of IONP and higher temperature increase the rate and decreases the vaporization threshold of PFC emulsions.
Chapter I: Introduction

Ultrasound technology has a variety of applications in both clinical and non-clinical settings. The relatively inexpensive, portable, safe, and real-time modality of ultrasound (US) has made it one of the most popular methods of medical imaging in clinical diagnostic and guided surgeries over the past 10 years [8].

There are over 75,000 ultrasound instruments installed in the United States, compared with only 7,000 instruments for computed tomography and 5,000 for magnetic resonance imaging [8]. In addition, diagnostic and therapeutic applications of US in cancer patients have introduced new uses and challenges for this technology. Although US continues to experience new technical innovations, the fundamental aspects of US imaging—including the basics of ultrasound physics, interactions of ultrasound waves and tissue, ultrasound pulse formation, scanning of the ultrasound beam, echo detection, and signal processing—remain unchanged.

1.1 Basics of Ultrasound Physics

Sound waves are pressure or mechanical waves that result in the movement of the particles of a medium about their mean positions. Ultrasound waves also consist of mechanical waves and have frequencies above the upper auditory limit of 20 KHz. These waves need to travel through some physical
medium such as air, water, or tissue, and they create regions of high and low pressure amplitude relative to the ambient pressure as they travel. This movement can be described mathematically by the wave equation:

\[
A = A_0 \sin(2\pi f t)
\]

In the case of medical US, these waves are longitudinal waves with a frequency range of about 2–15MHz [18]. The longitudinal component of ultrasound waves causes oscillatory motion of media in the direction of wave propagation and creates higher penetration depth for imaging purposes. On the other hand, the transverse component of ultrasound (shear waves) rapidly attenuates in tissue and does not play a direct role in the final output or ultimate image formation [12].

The velocity of sound is determined by the rate at which the wave energy is transmitted through the medium. Ultrasound waves travel with different speeds in different media; this differentiation of wave propagation speed is the basis of image formation in ultrasound imaging modality. Table (1) shows velocities of US pulses through different biological tissues. Since the speed of sound is inversely related to density and compressibility of the medium, the velocity of US waves is maximum for more rigid structures such as bone and minimum for less rigid structures such as fat and kidney tissue. [17].
Table 1: velocity of ultrasound waves in different biological tissues [2]

<table>
<thead>
<tr>
<th>Biological Tissue</th>
<th>Fat</th>
<th>Amniotic Fluid</th>
<th>Kidney</th>
<th>Muscle</th>
<th>Bone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Velocity (m/s)</td>
<td>1,450</td>
<td>1,540</td>
<td>1,464</td>
<td>1,600</td>
<td>4.080</td>
</tr>
</tbody>
</table>

Therefore, given the velocity of sound in the tissues $c$ and the time it takes for sound to travel in the body $t$, equation (1) can be used to compute the depth $d$ of the site that detected echoes produced.

\[
(2) \quad d = \frac{ct}{2}
\]

The $\frac{1}{2}$ factor in this equation results from the fact that time $t$ is the time from the pulse generation to echo detection, which includes the travel time of the pulse from the transducer to the reflector and back to the transducer.

Another fundamental property of US waves is the wavelength ($\lambda$), which is defined as the distance between the repeating units of the waveform and is related to the frequency and the speed of the sound by equation (3)

\[
(3) \quad c = f \cdot \lambda
\]

As this equation suggest, since the frequency of sound is constant, going from a medium of one acoustic velocity to another with a different acoustic velocity, the wavelength has to change. In biological tissues, US pulses may have a broad wavelengths ranging from 0.77 mm at 2 MHz transducers to 0.10 mm at 15MHz transducers [10]. The ultrasounds frequency and wavelength are important factors in determining the resolution of ultrasound images and the depth of US penetration into biological tissues. The choice of frequency
determining factor for the spatial resolution of the image and imaging depth: lower frequencies produce less resolution but image deeper into the body and vise versa. Superficial structures such as muscles, tendons, testes, breasts, and the neonatal brain for example, are imaged at a higher frequency (7-18 MHz), which provides better axial and lateral resolution. Deeper structures such as the liver and kidney are imaged at a lower frequency (1-6 MHz) with lower axial and lateral resolution but greater penetration [12].

Intensity ($I$), or the power per unit cross-sectional area, is another important acoustic property of ultrasound waves and is measured in $W/m^2$. Acoustic waves carry energy. If a surface with area $S$ perpendicular to the direction of the wave propagation is identified, then at every second a certain amount of energy will pass through the surface, which is called power and is measured in Watts (W). The intensity of US is proportional to the pressure amplitude, and particle displacement amplitude, or particle velocity amplitude [17].

Ultrasound that is highly concentrated or focused has a higher intensity than ultrasound emitted with the same power but spread over a broader area. High intensity focused ultrasound (HIFU) machines used in a variety of clinical applications such as tissue ablation for tumor treatments, hyperthermia, and activation or enhanced drug delivery make use of this concept. In this project, as will be discussed in chapter 2, HIFU has been used to generate high ultrasound pressures to vaporize perfluorocarbon emulsions.
1.2 Interaction of US energy and Matters

Interaction of US with matters, including biological tissues, can be divided into 2 general categories, macroscopic interactions and microscopic interactions. In the following section each one of these categories will be reviewed in more detail.

1.2.1 Macroscopic Interaction of US Energy and Matters

Interaction of US waves with matters in macroscopic scale is very similar to the wave behavior observed with light and includes reflection, refraction, scattering, diffraction, interference, and absorption. All except interference reduce the intensity of the beam, which is termed attenuation.

The major interaction of interest for diagnostic ultrasound is that of reflection. Reflection happens off of specular reflectors, which are defined as interfaces with dimensions greater than one wavelength in diameter, and is the major factors for organ outlining in diagnostic ultrasound.

Generation of echoes in ultrasound is due to the intrinsic properties of material called the acoustic impedance ($z$). As shown in the equation (4), this property depends on the speed of sound ($c$) and the density of the material ($\rho$).

$$ (4) \quad z = \rho \cdot c $$

Pulses that are generated with an US transducer will travel in the medium and interact with a surface. In biological systems, when an incident ultrasound pulse encounters an interface between two types of different tissues with
different acoustic impedance values, it will create an echo that can later be
detected by an US transducer. The incident of transmitted pulses with these
specular reflectors results in partially reflected echoes and partially transmitted
pulses deeper into the medium. As the reflected echo pulse travels back in the
medium toward the transducer, part of the energy dissipates and therefore its
intensity decreases. The intensity of the reflected echo as a function of the
incident intensity depends on the difference in acoustic impedances of the
chosen medium and can be calculated using the equation (5):

\[
I_r = \left( \frac{Z_2 - Z_1}{Z_2 + Z_1} \right)^2 I_i
\]

where \( I_r \) is the intensity of the reflected pulse, \( I_i \) is the incident intensity, and \( Z_1 \)
and \( Z_2 \) are the acoustic impedances of the media across the interface. The
difference in \( Z \) is commonly referred to as the impedance mismatch between the
two media. The greater the impedance mismatch, the greater the percent of
energy that will be reflected at the interface or boundary between the two media.
This will become critically important later in our design, as we were required to
minimize the impedance mismatch between our imaging phantom and its
surrounding medium. In body tissues, for example, some of the strongest echoes
are those generated by a muscle-fat interface (\( I_r=0.015 = 15\% \)), whereas a pure
liver-kidney interface generates much weaker echoes (\( I_r=0.0004 = 0.04\% \)) [7].
Another interaction of US beams and tissue is refraction, which causes beams to bend at the interface between two materials. This bending occurs because a portion of the wavefront travels at a different velocity at the second medium. However, as the velocity of US beams is almost constant in biological tissues, refraction does not pose any problems. Under certain conditions it might, however, be a major cause of image artifacts and be responsible for incorrect object shape [17].

When dimensions of particles \( d \) are smaller than the ultrasound wavelength (i.e., \( d << \lambda \)), scattering, or non-specular reflection, is an important interaction between US and tissue. In this case a wide angle of scattering occurs and echo intensity is greatly reduced. The scattering strength of these structures is proportional to the sixth power of the particle radius and to the forth power of the ultrasound frequency [9]. In the case of imaging small objects such as blood
cells in the body, scattering can provide the majority of ultrasound information [17].

Other important factors that can effect the attenuation of incident US intensity are diffraction and absorption. Absorption is the only process by which sound energy is dissipated in a medium; all other modes of interaction decrease the US beam intensity by redirecting the energy of the beam. Absorption is the process in which US energy is transformed into other energy forms, primarily heat, and has been primarily used in therapeutic ultrasound.

Both absorption and scattering are strongly dependent on the frequency and the propagation distance of sound waves. Therefore the US attenuation is inversely proportional to the traveling distance of the sound wave and the frequency of US pulses. Although attenuation of US in soft body tissues varies, for the most part it falls in the range of about 0.3–0.8 dB/cm/MHz [13]. Therefore, lower ultrasound frequencies are required to ensure that enough ultrasound energy will be received by structures deep in the body. However, as mentioned in section 1.1, there exists a fine balance between the frequency of incident waves and the resolution of US images as they are inversely related.

### 1.2.2 Microscopic Interaction of US Energy and Matter

Three mechanisms by which ultrasound interacts with matter on a microscopic scale include thermal, cavitation, and mechanical interactions.

As the US beam travels through the medium, the intensity of US decreases and the sonic energy is absorbed and converted into heat. This
phenomenon is due to the thermal interaction between the US energy and the molecules of the medium. The rate of temperature rise depends on the temporal average intensity, the absorption coefficient of the medium, and the heat-transport process [17].

As the US wave propagates through the medium, regions of compression and rarefaction are created. Thus localized regions in the medium are subjected to an increase and decrease in pressure in an alternating fashion. These pressure changes can cause gas bubbles to form and/or grow and exhibit dynamic behavior. This phenomenon is known as cavitation and can be in either a stable or transient form [17].

In stable cavitation, the microbubbles already in the medium expand and contract during each cycle in response to the applied pressure oscillation. The bubbles also grow in size as dissolved gas comes out of solution during the negative pressure phase [17]. Transient cavitation on the other hand, is a more violent form. During the rarefaction phase, bubbles are formed by dissolved gases coming out of the solution. The change in pressure during the compression phase causes the bubbles to collapse completely, producing shock waves [17].

There is a general consensus that cavitation is a threshold effect. For example the threshold for transient cavitation in water is 300 W/Cm$^2$ at 1 MHz and increases at higher frequencies [21]. The pulse sequence may have considerable importance particularly for stable cavitation, which appears to be a resonant effect acting over several cycles.
The final mechanism of US action on matter is mechanical. The US wave is propagated through the medium by interactions between the neighboring particles. The particles undergo considerable change in velocity and acceleration. As such, an object of different density than that of the surrounding medium will experience a torque in the ultrasonic field and may create mechanical motion.

1.3 Ultrasound Transducers

Transducers are the components in the ultrasound machines that are responsible for generating the incident wave and sensing the reflected wave. The active element in the heart of these transducers is a piece of piezoelectric material with electrodes attached to two of its opposite faces. This material is partially charged and responds to any change in an external electric field across its electrodes by aligning itself with the electric field, resulting in induced dipoles within the molecular or crystal structure of the material. This alignment of molecules causes the material to change dimensions and generate mechanical waves. The reverse of this process is also true. By applying mechanical force, some piezoelectric material can generate electric fields.

Piezoelectric material inside the ultrasound transducers receives a short electrical pulse and converts that to a corresponding pressure wave pulse. The pulse waves propagate down through the tissue and away from the transducer. The tissue then absorbs, scatters, reflects, and refracts the wavefront. The reflected waves travel back to the transducer as they become more scattered,
reflected, and absorbed through the tissue. As the transducer switches to the “receive” mode, it converts the pressure waves to electrical pulses and generates the image displayed on clinical ultrasound scanners.

1.4 Phase-Inversion Harmonic Imaging

During the past decade, significant advancements have been made to basic imaging approaches, which in turn have provided us with higher quality images and broader applications for this modality. One of these advancements is tissue harmonic imaging. The term *harmonic* refers to frequencies that are integral multiples of the fundamental frequency, for example, the second harmonic, which has a frequency of twice the fundamental frequency.

Higher harmonics can be created by non-linear scattering, e.g. from gas microbubbles, or by non-linear propagation of the ultrasound pulse. When harmonic B-mode imaging is used to improve image quality and contrast resolution of tissues, the technique is called tissue harmonic imaging (THI). As will be discussed later in chapter 2, this mode of imaging is used for microbubble detection in this project.

Harmonic imaging is performed by transmitting at one frequency and receiving at twice that frequency. Echoes from the tissue reflect at the transmitted frequency, while those echoes arising from contrast agents are at higher harmonic (2
\(^{\text{nd}}\) harmonic) frequencies. The result is a fairly strong signal
from the contrast medium in blood and a suppressed signal from surrounding tissue.

As US waves travel deeper in the tissue, they become more and more distorted. These distorted waves contain frequency components centered around higher-order harmonics. Thus, imaging these higher-order harmonics can be advantageous since it preferentially enhances the echoes from ultrasound contrast relative to the surrounding tissue, allowing improved visualization of blood flow. Harmonic imaging also reduces imaging artifacts that occur in conventional two-dimensional echocardiography.

Another approach to removing the fundamental frequency from the received echo spectrum uses the mathematical relationship between the phases of the fundamental frequency and higher order harmonics. Phase-inversion technique, on the other hand, utilizes multiple transmitted pulses that vary in phase. While this phase difference is maintained on fundamental “transmit” and “receive” pulses, harmonic signals generated by the tissue have a different shape and phase than those of the transmitted pulse. Therefore, by summing the received pulses, the fundamental frequencies cancel out due to destructive interference, and harmonic signals add up to generate a stronger outcome.

In summary, with phase cancellation techniques, two pulses are transmitted, and then the received echoes are combined in a buffer within the scanner memory. The fundamental frequency bands cancel because they are 180° out of phase. However, the second harmonic of the original pulse and its mirror image are in-phase and add constructively. An image formed from these
two cycles consists solely of echoes from the in-phase second harmonics, with the fundamental band removed through phase cancellation [25]. Therefore, the resolution and sensitivity of harmonic imaging can be further improved by use of the phase-inversion technique.

![Diagram of pulse-inversion harmonic imaging](image)

**Figure 2:** Basic physical concept behind pulse-inversion harmonic imaging [13]

### 1.5 Microbubbles as US Contrast Agents

Contrast agents alter image contrast in a specific way to provide more information and enable the diagnostician to distinguish between normal and abnormal tissues [19]. Most contrast media rely on differences in acoustic impedance between the agent itself and the surrounding medium. Enhancing the power of the backscattered echo signal is the most important function of an ultrasound contrast agent [19]. The received ultrasound intensity, $I_s$, is the
function of the incident intensity, $I_i$, and the scattering cross section (in m$^2$) of the reflector according to equation (6) [21].

$$I_s = \frac{I_i \sigma}{4\pi R^2}$$  

Where the $\sigma$ is the scattering cross section and $R$ is the distance between the transducer and single small scatterer. The scattering cross section for small reflectors depends on differences between material properties of the scattering and surrounding medium and since the acoustic properties of air are so different from those of blood, microbubble-based ultrasound contrast agents can enhance reflected US signals significantly and generate high quality images. Another mechanism to echo enhancement also exists. Microbubbles (MB) have resonance frequencies coinciding with those used in diagnostic imaging (1 to 10 MHz) [19]. Therefore upon their exposure to the compression and expansion forces of a sound wave at diagnostic ultrasound frequencies, they can resonate and become transmitters themselves. Equation (7) can be used to estimate the resonance frequency of a bubble acting as a harmonic oscillator

$$F_0 = \frac{6500}{d}$$  

Where $d$ is the diameter of the bubbles in µm [25]. The most useful scattering sizes range from 3-8 um given the frequencies used in medical ultrasound [19]. However, due to the need to stabilize bubbles, which dampens the oscillation, the resonance frequencies measured in practice are somewhat higher than predicted.
Microbubbles are therefore, not only produce a passive backscattering, but also actively transmit due to their own oscillations. The signal amplitude from MB oscillation is governed by a principle first described by Rayleigh and Plesset for free bubbles [21]. This signal is dependent on the compressibility and density of the gas inside the bubble, the viscosity and density of the surrounding medium, the frequency and power of the ultrasound applied, and bubble size [11]. The backscattering of MB agent will also depend on pressure effects. The pressure within an air bubble can be calculated via the Laplace equation:

\[ \Delta p = \frac{2T_s}{r} \]

where \( T_s \) represents the surface tension of the bubble and \( r \) is the radius. Increased static pressure will result in a drop in backscattering from a microbubble agent. Also, there is a decay of backscattering of a contrast agent over time, due to the diffusion of gas back into the blood and the subsequent breakdown of bubbles [11].

It has been shown that the higher the elastic modulus of bubbles and the larger the bubbles, the higher the scattering intensity. Therefore to maximize the resonant and scattering intensity, bubbles need to have a soft elastic shell and have a higher radius [7, 16]. However, there is a fine balance between the elasticity of MB’s shell, and its stability and half-life in the body. As several minutes are needed for an effective and convenient examination of a patient, an adequate in vivo bubble half-life is required. Consequently, the air bubbles have to be stabilized with an outer shell in order for the agent to achieve sufficiently long persistence. In addition, an acceptable upper size limit for in vivo
applications is determined by the necessity for bubbles to cross capillary beds; thus, this limit must be in the range of 1–7µm.

A variety of MB contrast agents of different shell and gas-core makeup are currently available. Most of MBs’ shell material is composed of albumin, galactose, lipid, and polymers [36]. Microbubble cores may contain regular atmospheric air gas or heavy gases such as perfluorocarbon or nitrogen. The determinant of the persistence of the microbubbles in blood is shown in the equation:

\[ T = \frac{r_c \rho}{D C_s} \]  

where \( r \) is the radius of the bubble, \( \rho \) is the density of gas, \( D \) is the diffusivity of gas, and \( C_s \) is the saturation concentration. Thus, the longevity of a bubble is directly increased by its radius and the density of the gas inside. Persistence is inversely related to or decreased by the diffusivity. It is clear that the persistence of a microbubble contrast agent can be prolonged by increasing either the size of the bubble or density of the gas, or by decreasing either the diffusivity or concentration of saturation of the gas. Since fluorocarbon gases have higher density, decreased diffusivity, and lower concentration of saturation than room air, they increase bubble persistence. In addition, the gases themselves are well tolerated by humans.

1.6: Properties of Perfluorocarbon
Perfluorocarbons (PFCs) are a class of compounds that are primarily composed of carbon and fluorine atoms. PFCs like oil are immiscible with water and cannot be given intravenously unless emulsified. There are different members in the family of PFC with unique properties. For example, Perfluorooctyl-bromide (PFOB), a PFC in which the terminal fluorine is replaced by bromine, is radiopaque.

PFCs are inert compounds that have low surface tension and very low toxicity when ingested or inhaled and have a broad range of vapor pressure. The length of time that they remain in the body is related to their molecular weight, vapor pressure, and fat solubility. Lower MW and fat soluble PFC molecules have shorter body half-life.

PFC emulsions when injected intravenously can act as contrast agents. The echogenic effect of PFCs is due to their higher density (1.9 g/ml) and lower acoustic velocity (600 m/sec) than tissues [19]. Although PFC emulsions scatter US relatively better than water, they are in general poor scatterers, especially in comparison to MBs, which are larger in diameter and much more elastic [19].

Unfortunately because the reflectivity of PFC emulsion particles is limited, relatively large volumes are required to produce enhancement [39]. Therefore, it is clear that it would be beneficial if scattering of a microbubble system could be achieved without sacrificing the many advantages of emulsion.

1.7 Dynamics of Microbubbles In-Vivo
As a result of the dramatic difference in the acoustic impedance of gas-filled MBs relative to the surrounding soft tissues as well as their high compressibility compared to that of any liquid or solid, microbubbles are $10^{10}$ to $10^{12}$ times more reflective than red blood cells [19].

The biggest challenge of using microbubbles as contrast agents in vivo is bubble stability and its half life time. When infused intravenously, the air dissolves very rapidly in the blood and thus bubbles are lost from the circulation before the ultrasound study can be completed. Several reasons can be identified to explain the shrinking of MBs in the blood. As shown in equation (10), the sum of gas pressures within the bubble can exceed the pressure of the gas in the blood, which creates a positive pressure gradient towards the outside of the bubble and subsequently pushes the gas out

$$\Delta P + P_{Blood} + P_{atm} = P_{air} + P_f$$

where $\Delta P$ is the Laplace pressure. Additionally, oxygen metabolism lowers oxygen tension in the blood, which provides another route for oxygen to leave the bubble, and causes reduction in its size. Another reason for the short bubble half-life is the exposure to intense US energy leading to bubble destruction as bubbles rapidly contract and expand at resonance frequency.

Bubble half life can be increased by reducing the rate of solubility of their enclosed gas in the blood. This reduction can be achieved by replacing simple air gas with heavier perfluorocarbon (PFC) gases that are less water-soluble, yet volatile chemicals known for this application. However, injection of PFC vapor into the microbubble by itself is not sufficient to stabilize the bubbles in the blood.
[14]. Due to zero concentration of air within the bubble, they will create an osmotic pressure which will take up air from the blood and swell until the partial pressure of air inside the bubble equals the pressure of dissolved air in the blood.

When insufficient PFC is present, the bubble will shrink rapidly and may eventually reach the collapse radius. Therefore, the main design requirement for MBs to be osmotically stabilized is to ensure that the partial pressure of the PFC is set to exactly counterbalance the Laplace and arterial pressures [14]. In order to achieve this goal, the osmotic agent needs to have two important properties: low Ostwald coefficient value (<10$^{-4}$) and relatively high saturated vapor pressure at body temperature (>3 x 10$^4$ Pa) [14]. This way water-insoluble PFC vapor remains in the MB and dilutes the water-soluble gases so they have a partial pressure of 1 atm. This creates an equilibrium where the water-soluble gases can diffuse in and out of the bubble while the PFC vapor supports the surface tension and blood pressure forces [14,31].

1.8 Applications of Microbubbles:

The field of ultrasound imaging began in the 1960s and entered clinical application in the late 1980’s [3]. Advancement in the fabrication of more stable MBs to gain a better understanding of the interactions between sound waves and the surface of MBs led to discoveries of other diagnostic and therapeutic applications for ultrasound technology.
As an imaging tool, contrast agent can be used for vascular imaging to determine any occlusion, abnormalities and flow pattern in vessels. This is one of the most promising applications of MBs and takes advantage of the fact that rheology of MBs in the microcirculation is nearly identical to that of red blood cells (RBCs), and also US energy is able to destroy MBs at high transmitted acoustic power [1].

MBs can also be used to address the problem of distortion in ultrasound imaging [16,13]. Attenuation of sound energy as it passes through tissues with different attenuation coefficients and varying speed of sound can cause loss of contrast and resolution in ultrasound images. This, for example, is more pronounced in the case of brain imaging where US needs to pass through the skull layers, which has very high acoustic impedance [25,13]. One way to correct for this aberration is through point-target techniques. This technique relies on the existence of a sparsely distributed point source in the region-of-interest (ROI) to correct for the attenuation artifacts. Studies have demonstrated that ultrasound induced cavitation of MBs within the tissue can be used as point-targets for image aberration correction [1,6].

In the area of diagnostic applications, US contrast agents can be used to evaluate microvascular perfusion and tissue blood flow [30,13]. Tumor growth requires an extra source of blood flow into the cancerous tissue. Therefore, this technology can be used to characterize microvascular perfusion as microbubbles fill the organ in real time and enhance tumor detection using US. Real time monitoring of filling pattern by MBs and using contrast specific US imaging allows
for not only detection of tissue abnormalities but to also improve the ability to characterize diseases by distinguishing, for example, between infection, infarction, trauma, and cancer [8].

Molecular imaging is also another important diagnostic application of US contrast agents [33]. Microbubbles coated with specific targeting agents have been used to attach to a specific molecule of a given pathology, which then can be imaged using ultrasound. Targeting can be achieved by attaching appropriate ligands to the surface of microbubbles [33,5]. One technique for binding contrast microbubbles to a target is by modification of the contrast agent shell. However, since this strategy limits variability with regard to target structure, a better method would be to use antibodies, glycoproteins, carbohydrates, peptides, or other molecules attached to microbubbles. This can be done by covalent bonding or through avidin-biotin coupling mechanisms [33,8].

In contrast to the commonly used microbubbles, the exceptional properties of liquid perfluorocarbon (PFC) emulsion nanoparticles enable this contrast agent to exit the intravascular space and acoustically identify extravascular targets [7].

Microbubbles have also been greatly studied for therapeutic applications. For example, Boehm et al. (1997) have shown MBs restrict blood supply to cancer cells, and hence starve them to death [6]. Up to now, different materials and procedures have been used to accomplish this purpose. These materials have been absorbable, such as blood cloth and gelatin sponges, or non-absorbable such as balloons and coils. A safer, more convenient, and more controlled method to target specific regions for occlusion is through injection of
microbubbles to those targeted sites [6]. As will be discussed later in this report, by injecting perfluorocarbon (PFC) emulsions instead of MBs directly, ultrasound energy can be used to acoustically vaporize emulsion droplets to produce microbubbles and occlude multiple vessels or a capillary bed at a specific target site. This phase conversion of liquid emulsions into MBs is called acoustic droplet vaporization (ADV).

A couple of other clinical applications of MBs include contrast-enhanced US images taken at different stages of therapeutic process, which can be used to monitor the healing progress and efficacy [31]. Also, the acoustic power needed to achieve sonoporation used to produce transient enhancement in the permeability of cell membrane, appears to be substantially reduced when microbubbles are present [9].

1.9 Limitations of Current Technologies

Microbubbles are good targeting carriers for on demand delivery of diagnostic and therapeutic agents such as drugs. However, due to their 1-5 µm diameter size they are not able to pass through endothelial cells and therefore their application is essentially limited to pathologies that express specific antigens within the vascular lumen [29]. In addition, MBs have limited carrying capacity for drug delivery and limited circulation life in the body for targeting applications making them less efficient delivery and imaging agents [29, 13].

In contrast to the commonly used microbubbles, PFC emulsions are relatively smaller in size, allowing them to exit the intravascular space and target
a broader range of cellular structures. Also, their higher carrying capacity and particle count improve the efficiency of MB applications [29,13]. Despite all their advantages emulsions are poor scatterers and therefore are not appropriate as US contrast agents. One way to overcome this issue is to convert the emulsions to MBs using US as they arrive at their target site. However, low boiling point and larger size are required for emulsion to achieve vaporization, which can introduce new challenges and limit their in vivo applications.

Several studies reported the administration of low boiling point (29°C), micro-size PFC droplets in animal models in which their spontaneous vaporization into MBs caused occlusion of vessels in sites other than the targeted area [7,11,18]. While some studies used more stable and smaller PFC droplets in their experiments, they reported higher ultrasound power to achieve acoustic vaporization, which is not clinically feasible [4, 7,18].

Therefore, we hypothesize that using higher temperature and IONP as nucleation sites within the emulsions can lower the US energy required to vaporize the submicron-sized and more stable PFC droplets. This on the other hand will prevent spontaneous vaporization of emulsions in vivo.

1.9.1 Why Iron-Oxide Nano particles?

The iron oxide magnetite (Fe₃O₄) is the most studied and most commonly available material due to biocompatibility and suitable magnetic properties. IONP are small particles typically in order of 5~20 nm in size, have superparamagnetic properties, and therefore exhibit no overall magnetic hysteresis. Also due to
fluctuation of their magnetic moment with thermal energy at a given temperature, they exhibit no remnants [5].

The magnetic hysteresis behavior of ferro-magnetic or ferrimagnetic particles in a time-varying externally applied AC field produces magnetically induced heating per unit volume. Although magnetically hard materials can generate more heat, they require strong AC fields [35]. Therefore, magnetically soft materials have an operational advantage because of the ease of reaching a high magnetization state with relatively low, practical, and available AC fields, but their tendency to aggregate due to their single-domain, and high-coercivity magnets property make it difficult to manipulate them or administer them intravenously. Therefore iron oxide was chosen because of its small size and efficient heating.

1.10 Aims of the Thesis:

The objective of this study was to investigate the effects of temperature and the presence of ironoxide-nanoparticles inside PFC emulsions on the threshold of ADV, and the phase conversion rate from liquid emulsions to gas filled microbubbles. In order to achieve this objective, four main specific aims were defined: 1) Control the local temperature of PFC droplets with and without IONP while sweeping over a broad range of temperatures from room temperature to several degrees above the boiling point (superheated state), 2) Apply different ultrasound powers to the emulsions to convert them into microbubbles 3) Detect the microbubbles as they leave the system and 4)
Analyze the data to determine the threshold and rate of droplet vaporization as a function of temperature and HIFU power
Chapter II: Experimental Study

2.1 Introduction

Ultrasound as a convenient, safe and relatively inexpensive imaging modality has been used for different diagnostic applications. Recently, US has become of interest as a therapeutic tool in applications such as interventional cardiology and non-invasive induction of thrombolysis [2]. Development of microbubbles as ultrasound contrast agents was a significant step forward to improve the quality of US images and generate more potential applications in therapeutic areas for this imaging modality.

Microbubbles are small gas filled microspheres with specific acoustic properties that make them useful as a contrast agent in US imaging. More advanced materials used for MBs, improved their functionality to make them more stable and controllable for in vivo applications. Replacing regular air gas to more stable and osmotically more favorable heavy gases such as perfluorocarbon gas inside the bubbles and substituting its thin lipid shell to a more advanced coating improves their applications and increases their practicality in clinical settings [5,7]. In addition, more advanced US machines with improved image processing features can increase the US sensitivity to these contrast agents and improve the signal-to-noise ratio. For example, oscillation of microbubbles at the fundamental frequency of the US transducers generate
reflected signals with frequencies in multiples of the fundamental frequency, which can be received by harmonic imaging modalities and analyzed for better quality images.

Despite the higher reflectivity of microbubbles, their short circulating time in the body limit their in vivo applications and call for an alternative form of contrast agents. Studies have been shown that as an alternative to MBs, it is possible to use liquid PFC emulsions. PFC droplets are more stable than MBs and their size is orders of magnitude smaller, which give them more circulation time and make them superior for extravascular targeting [4,17,22]. However, their application in vivo as contrast agents presents some limitations. Unlike microbubbles that are $10^{10}$ to $10^{12}$ times more reflective than red blood cells, PFC droplets are only 7 times more reflective than cells in normal tissue and do not generate harmonic signals [4,6]. In addition, PFC emulsions do not undergo stimulated acoustic emission processes in which, high US pressure destruct MBs and generate a high intensity, wide spectrum, ultrasound signal and cause the released gas to dissolve in the surrounding fluid [11].

Therefore, to be able to use the advantages of liquid emulsions and overcome their limitations, it is desirable to vaporize droplets and convert them into microbubbles at the target site. The technique used to vaporize the liquid droplets into gas bubbles by using US acoustic energy is called Acoustic Droplet Vaporization (ADV).

Currently, several different formulations of PFC emulsions have been tested. The main challenge with most of these formulations has been the
spontaneous evaporation of low boiling point emulsions (b.p < 50°C) upon their administration in vivo. To address this problem, more stable emulsions with higher boiling points (>50°C) has been formulated. These agents need to be stable against US scanning, up to an acoustic pressure threshold above which the agent can be vaporized intentionally [13].

Increasing the boiling point and reducing the size of the emulsions to submicron droplets improves the visualization of the target since US will detect droplet activation rather than MB accumulation [21]. It also allows for extravascular and intravascular targeting and higher droplet accumulation on the surface of cells, which will generate higher intensity signals [3]. However, these submicron and more stable emulsions require higher US power to vaporize to MBs.

We hypothesize that by combining the effect of higher temperature and using IONPs as nucleation sites, the rate of emulsion conversion to MBs can be increased and the threshold of US energy required to achieve droplet vaporization can be significantly reduced.

2.2 System Design

Objective of this project was divided up into three specific aims: To control and adjust the temperature of emulsions with and without encapsulated IONP, to apply a range of US powers to determine the acoustic threshold of droplet vaporization, and the conversion rate of droplets to microbubbles, and lastly to detect and quantify the MBs produced at different temperatures and pressures.
for emulsions with and without IONP. Therefore, a list of design requirements was put together to achieve these goals.

2.2.1 Design Requirements:

To allow for vaporization of droplets under controlled temperature and ultrasound power, the following system requirements needed to be satisfied:

- A heating pump had to ensure a constant and homogenous temperature gradient around the emulsions
- An ultrasound system was required to deliver a broad range of US pressures to a 2 to 3 mm$^2$ area. Since the pressure required for droplet vaporization increased with a decrease in US frequency, higher frequency was more desirable for this system.
- An ultrasound scanner with harmonic imaging was required to detect the microbubbles
- A chamber was required to allow for the interaction of US waves and emulsions. This compartment, which was referred to as the interaction chamber, had to have an appropriate geometry and material property to minimize wave scattering and attenuation. It also had to provide a 2 to 3mm$^2$ of interaction area between the US waves and emulsions to limit the number of droplets that interact with US energy and to maximize the effect of applied pressure.
- A water bath was required to control and adjust the temperature of emulsions by homing the emulsions to expose them to the hot water
produced by the heating pump. The material and geometry of the hot water bath had to ensure minimum attenuation of the HIFU US intensity and minimum heat transfer (dissipation) to the surrounding medium (low acoustic attenuation coefficient and high thermal resistivity)

- An imaging phantom was needed to allow for the detection of microbubbles as they were leaving the interaction chamber. To ensure high quality images and accurate detection of microbubbles, the geometry and material of this phantom had to ensure the least US energy attenuation at high frequencies

- A flow pump with a controllable and laminar flow of emulsions with varying flow velocities was required to ensure a continuous flow of emulsions and MBs

More detailed specifications on some of the systems that were used in this process is described in the conclusion section.

### 2.2.2 System Specifications:

As outlined in section 2.2.1, the following components were utilized to satisfy the design requirements; a HIFU system (ExAblate 400, Insightec, Dallas, Texas), a continuous flow pump (ISCO pump 100DX, Teledyne ISCO Inc., Lincoln, NE), a HIFU interaction chamber (Advanced Polymer Inc., NH, USA), a water bath and a heat exchanger unit (NESLAB recalculating chiller, ThermoScientific, Waltham, MA), a US imaging phantom, and a clinical US imaging system (ACUSON Sequoia, Siemens, Mountain View, CA)
1. High Intensity Focused Ultrasound (HIFU)

A high intensity focused ultrasound (HIFU) machine was used to provide the necessary acoustic energy to vaporize the emulsions. ExAblate 400 was manufactured for research purposes and is not commercially available and is consist of two main parts; transducer housing and computer unit.

1.1 Transducer Housing:

As shown in the figure (3), the housing is a hemispherical phased array transducer with 1000 single peizo-element which can be operate independently and generate a sharp focus (radius: 2.0mm in X/Y- and 3.0mm in Z-orientation) at the center of the transducer with central frequency of 0.22MHz.

1.2 Computer Unit

A computer unit, figure (4), controls the US electrical power, position of its focus, pulse repetition, and duty cycle.

The electrical power of the HIFU is the only parameter relevant to the US energy that can be interactively varied. Therefore, the change in the pressure at the focus of the HIFU was estimated using equation (11) while the electrical power of the machine was varied.

\[
P = \sqrt{\frac{P_{ac} \cdot Z}{A}}
\]

In this equation \(P\) is the pressure at the focus, \(P_{ac}\) is apparent acoustic pressure, \(Z\) is the impedance of the liquid, and \(A\) is the beam area at the focus. The apparent acoustic pressure is roughly 1/5 of the acoustic pressure delivered by HIFU system at its focus.
2. US Imaging System

Two clinical US scanning machines were used to detect the presence of microbubbles in the tube after they left the interaction chamber.

2.1 ACUSON Antares:

The ACUSON Antares ultrasound machine is a clinical scanning ultrasound system that was used in the initial experimental setup. This system operates under two imaging modes, general and tissue harmonic imaging (THI). As discussed in section 1.6, the latter technology has been used for contrast imaging and therefore, was more appropriate for detection of MBs in this work. The Antares system is compatible with a variety of transducers for different imaging applications. During this project, depending on the material of the imaging phantom, two of its linear transducers, VFX13-5 and VFX9-4, with central frequencies at 13MHz and 9MHz were used.

2.2 ACUSON Sequoia

Later experiments utilized the ACUSON Sequoia ultrasound machine, a standard clinical scanning system that provides a more advanced solution to contrast agent imaging applications. Similar to the Antares system, Sequoia also has two different imaging modalities such as cadence contrast pulse sequencing (CPS) and cadence agent detection imaging (ADI) technology. Both systems are discussed in more detail in the appendix A.1 and A.2. Cadence CPS technology has a unique ability to combine the nonlinear fundamental and higher order harmonic contrast signals to form a highly specific and sensitive contrast agent display. This system provided higher quality images for more accurate
quantifications of microbubbles compared to the Antares system. This unit was equipped with two transducers, a linear array transducer 15L8 with a central frequency of 15MHz and a convex transducer 4C1 with central frequency of 4MHz. The 15MHz transducer was used in this project to produce higher resolution images. The optimum parameter values for the gain, dynamic range, US power, and US frequency were highly dependent on the material of imaging phantom and are discussed in more details in the Chapter 3.

3. Continuous Flow Pump

As mentioned in the design requirement, a continuous, laminar flow of emulsions into the interaction chamber and microbubbles into the imaging phantom was required. In order to achieve this flow, the initial setup made use of a gravity fed tubing system to provide a column of emulsions with varying height to adjust the flow rate. Although this system could achieve laminar and continuous flow, it did not provide a precise control over flow rate or produce enough pressure difference to drive the emulsions and microbubbles in the tubing system.

As an alternative to this method, a flow pump was used to create a continuous flow of emulsions at a highly controllable fashion. The ISCO pump is a high precision, high pressure syringe pump which is capable of providing a continuous laminar flow from $1 \times 10^{-5}$ ml/min up to 50 ml/min. While higher flow rates were useful for flushing the system and cleaning the tubes, slower flow rate was used during the droplet vaporization and detection process.

4. Heating System:
Since one of our major control factors in the project was the temperature of emulsions, a system was designed and characterized to perform this task. This system was composed of two parts, a water bath and a heat exchanger unit.

4.1 Water Bath:

As shown in figure (5), a water bath was designed to heat the emulsions and adjust their temperature as the solution was circulating inside the HIFU interaction chamber. Sitting in the middle of the HIFU transducer housing unit, US waves had to pass through the hot water bath walls before they reached the interaction chamber. Therefore, the material and geometry of this unit was important to reduce the pressure attenuation and a shift in the location of the HIFU focus. In order to achieve this, a cylindrical shaped container made out of clear polyethylene with a relatively low acoustic attenuation coefficient and a good thermal conductivity of (0.5 W/mK) was used [10,4].

4.2 Heat Exchanger Unit

The NEALAB heat exchanger pump with a temperature range between -34°C to 135°C was used to create a homogenous temperature gradient inside the water bath. The temperature of water was closely monitored through the pump digital thermometer as well as a thermocouple that was placed inside the water bath. The pump had a temperature fluctuation of +/- 1°C and the difference between the readout value from the pump thermometer and the actual temperature of water bath was negligible.

5. HIFU Interaction Chamber:
One of the control variables in this experiment was the volume of emulsions that interacted with different US intensities at the focus of the HIFU. Large interaction chambers could expose extra emulsions to US waves at higher acoustic powers, which would introduce uncontrolled variable in the final result. Therefore, the dimensions of the interaction chamber had to be completely immersed in a 4 x 4 x 3 mm region in the focus of the HIFU.

Several materials and designs were used for the interaction chamber. The initial design used PDMS (Sylgard 184-, Dow Corning, Midland, MI), in a 1:10 curing ratio, to build a cylindrical phantom with a small (3 x 3 x 3 mm) cube in the bottom of the phantom. Inlet and outlet channels were placed in the PDMS phantom to allow for delivery of emulsions to, and recovery of generated microbubbles from the focus of the HIFU into the imaging site. This phantom was completely immersed in the water bath to adjust the temperature of emulsions. Although PDMS provided a solid and clear phantom and could tolerate high temperatures, its high attenuation coefficient and high thermal resistivity made it less than ideal material for this application.

As shown in figure (6), a U- shaped chamber was built out of two pieces of vertically oriented tubes (10 cm in height) which were connected via two L-shaped plastic connectors at the bottom and was fixed to the robotic mechanical arm on top. As discussed before, the geometrical dimension and material of the interaction tube had to ensure the minimum interference with the US energy to prevent any shift in the position of the HIFU focus and strength of the US pressure. Therefore, the chamber was made out of a transparent polyester tube
with inner diameter of 4.3 mm and a thin wall thickness of 0.07 mm which had a relatively low acoustic attenuation coefficient.

Emulsions entered the chamber from the descending tube and raised up the ascending tube where they interacted with the US waves. Given the flow rate of 5 ml/min from the pump and the dimensions of the tube, emulsions flowed with the speed of 0.5 cm/sec in the chamber which gave them about 36 sec to equilibrate with the water-bath temperature.

6. Imaging Phantom:

Detection and quantification of microbubbles was one of the most important and challenging aspects of this project. As mentioned before, a contrast specific US imaging system was used to detect the signals from microbubbles in the solution of emulsions after they left the interaction chamber.

Different designs and materials were used for the imaging phantom, out of which the last one generated the most accurate and high quality images. The first design was a 2 cm x 4 cm PDMS block with a 1 mm inner diameter channel in the center. Although transparency and rigidity of PDMS made it easy to work with and visualize the flow, its high attenuation coefficient generated low quality images with low signal-to-noise ratio.

The second phantom was a combination of 6% agar gel housing and a clear silicon tube with a 7 mm in diameter and a wall thickness of 1 mm, which was used for the proof of concept and parameter optimization experiments. Even though agar had a low attenuation coefficient, its maintenance, lower rigidity, and lack of tolerance for high temperature made it less desirable for this application.
As shown in figure (7), the final design was a piece of polyester tube, similar to the tube used for the interaction chamber that was continued upward from the focus of the HIFU and into a water tank from its bottom surface. The Sequoia transducer was placed parallel to the tube in the direction of flow of emulsions as it traveled from the interaction chamber to the waste container.

2.3 Materials and Methods

2.3.1 Production of Iron Oxide Nanoparticles Loaded Emulsions

Iron-oxide (Fe₃O₄) nano-particles (IONP) with diameters of about 5 – 20 nm cores and a hydrophobic coating of fluorinated surfactant perfluorododecanoic acid (PFDDA) was made prior to this project. IONPs were infused into the different emulsion formulations. Initial primary data suggested that PFC molecules containing Bromide (PFHB, PFOB and PFDB) can suspend highest concentrations of IONP. However, they also have a very high boiling point of greater 100°C. Since a boiling point between 37°C and 50°C was desirable, formulation of PFC emulsions with loaded IONP had to be optimized. Initial plan was to suspend the particles in PFHB emulsions and mixing different ratios of PFP and PFH (boiling point of 29°C and 53°C, respectively) to lower the boiling point.
60% w/v PFC emulsions with maximum concentration of IONP and a mean diameter of 200 nm were then made as follow: A solution of 25 ml of PFHB and 25 ml of PFH and IONP was added to a mixture of 0 gr –5.00 gr of egg yolk phospholipid (EYP) and 45.47 ml of PBS, while it was turaxed at high speed over a 5 minute time period. The mixed solution was then transferred to the Microfluidizer® processor (M-110Y Microfluidics, Newton, MA) where the solution made approximately 6 discrete passes with the maximum air possible (12,000–13,000 psig) to produce uniform IONP coated droplets. Each formulation was characterized by measuring its boiling point and particle size distribution and the optimum mixture with boiling point between 37°C to 50°C and size distribution around 100-150 nm diameter was selected.

**Boiling point:**

To determine the vaporization temperature of perfluorocarbon droplets, an undiluted 2 mL formulation of albumin-coated droplets was sealed in a 3 mL glass vial and vented with a 27-gauge needle. The vial was submerged into a water bath causing the water temperature to slowly rise. The boiling point was determined by visually monitoring the start of gas bubble formation in the liquid before it was vented through the needle. The experiment was conducted for a liquid PFC emulsions with and without IONPs.

**Emulsion size:**

A Zetasizer (Malvern instrument, Westborough, MA) was used to determine the size distribution of the emulsion samples with and without IONP. A Zetasizer uses light scattering technology to measure the hydrodynamic size of
nanoparticles. This device can measure a broad range of particle sizes from 1 nm to 10 µm in diameter. Emulsion samples were allowed enough time to equilibrate with room temperature before they were sized.

2.3.2 Acoustic Droplet Vaporization and Microbubble Detection

Vaporization of lower boiling point and micron size PFC emulsions have been shown using different frequencies and US intensities at room temperature [4,13,17,32]. Recently, Rowlands et al showed the vaporization of sub-micron (250nm) CdSe/ZnS quantum dot nanoparticles loaded PFC using a 1MHz pulse and up to 2MPa US pressure\(^1\). It has also been shown that the acoustic droplet vaporization threshold increases by decreasing frequency [25,32].

The HIFU system used in this experiment had a fixed frequency of 0.22MHz, which is smaller than any frequencies published on ADV. Also, the characteristic of emulsions with and without IONP was different from all the other published data. Therefore, a new range of US pressure, temperature, and emulsions dilution factor had to be established.

Different combinations of experimental designs and materials, emulsion formulations with different dilution factors, HIFU settings, and US imaging parameters were tested to vaporize the droplets and detect microbubbles. Some of these designs and formulations are discussed in the results section. Initial designs were simplified by eliminating the flow factor and water bath by

\(^1\) This work was presented during world Molecular Imaging Conference as an abstract and up to date there was no publication.
designing a less interactive imaging phantom and manually placing the interaction chamber in the focus of the HIFU. As such, the project was focused on producing a proof of concept and optimizing US parameters for both the HIFU and imaging scanners. Later designs, however, became more complicated with integrated flow, a temperature control water bath, automated positioning of the interaction chamber in the HIFU focus, and more automated imaging phantoms. Although the output of initial experiments did not provide any quantitatively meaningful data, this setup allowed fine-tuning of HIFU parameters such as repetition frequency, number of pulses, and duty cycle. It also helped optimize the quality and accuracy of images captured. In addition, these experiments were critical to optimize the PFC/IONP formulation and adjust emulsion size and boiling point.

2.3.2.1. Proof of Concept and Parameter Optimization

A simplified setup was used for the proof of concept and optimization process. 3 ml PFP emulsions were diluted with 30 ml DI water (1:10 ratio). 2 ml of this solution was gently injected into the interaction chamber. The chamber was made out of a silicon tube with an internal diameter of 7 mm and a wall thickness of 1 mm and was formed into a 70°C arc to ensure minimum interference with US energy. Both ends of the tube were completely sealed to prevent air bubbles from entering the solution.

The chamber was then manually placed in the focus of the HIFU where different combinations of repetition frequency, duty cycle, and US power were
delivered to the emulsions. The tube was then placed in an agar imaging phantom and an Antares imaging system was used to detect microbubbles inside the tube. The imaging phantom was made out of 6% agar gel to minimize the attenuation of US and increase the quality of images.

The effect of temperature on the ADV threshold and the conversion rate was also determined. 3 ml of PFP emulsions was heated to a range of temperatures which was then gently injected into the interaction chamber. The US power and sonication setting were held constant while the temperature changed from 25°C room temperature to 50°C, which was 20°C superheated. The interaction chamber was then placed in the focus of the HIFU machine and MB concentration was imaged and compared at different temperatures. To ensure that heating of the emulsions to higher temperature was not the cause of droplet vaporization, the chamber was imaged right after heating and before the application of HIFU.

Although two ends of the tube were sealed to prevent mixing of air bubbles with the solution, pure water was used as a controlled case. To ensure that the bubbles were due to the emulsions and not mixing of air with the solution, the same set of experiments were repeated using pure water instead of liquid PFP droplets. The outcomes of these experiments are presented in the results section.

2.3.2.2 Acoustic Droplet Vaporization under Controlled Conditions
Although the basic design setup and experiments were crucial for the proof of concept and optimization of US parameters and emulsion formulation, they did not provide any quantitative data or conclusion for the effect of temperature and IONP on the rate and threshold of ADV. To obtain a more quantitative result, a more complex and complete system was required. This system had to incorporate the flow of emulsions in and out of the interaction chamber, real time temperature adjustment of emulsions, and real time monitoring of the microbubbles production. In addition, the final system had to provide a means to control the US power, volume of emulsions in the focus zone, and time delay between the vaporization and microbubble detection.

As shown in figure (8) a system was designed to control all the necessary variables and provide a more accurate means for quantitative measurement of the effect of temperature and IONP on the threshold of ADV and rate of droplet conversion.

To reduce the concentration of emulsions for in vivo applications, high dilution factors, on the order of 1:1000 to 1:10000, have been used before. However, to ensure the optimum effect of US energy in this project, concentrated PFP emulsion samples were diluted by adding 8 ml of the droplets to 160 ml of saline (1:20 ratio). The ISCO pump was then filled with 103 ml of the diluted PFP emulsion and was used to pump the solution into the system. The flow rate of the pump was set at 5 ml/min with a given tube diameter $d$. Assuming a laminar flow condition ($Re < 2300$), the flow velocity of 0.5 cm/sec, was achieved $Re = \frac{\rho d u}{\mu}$.
where $\rho$ and $\mu$ are density and viscosity of the host liquid, respectively, and $U$ is the flow speed.

The schematic of the experimental setup, figure (8), shows different components of the system. The pump was connected to the interaction chamber through a series of stainless steel tubing. The interaction chamber was placed inside the water bath to adjust the temperature of emulsions. The NEZLAB heat exchanger unit was used to create a homogenous temperature gradient inside the water bath and monitor the temperature in the system. The water bath was ultimately placed in the center of the HIFU transducer housing, while the automated mechanical arm was used to position the interaction chamber in the water bath so that the top portion of its ascending tube sat at the focus of the HIFU.

The output of the interaction chamber was then continued upward to guide the emulsions and gas bubbles into the imaging phantom. A linear array ultrasound (nominally 15 MHz) was connected to the Sequoia diagnostic scanner and was placed parallel to the flow and aligned with the tube to detect the production of microbubbles.

A 5 ml/min continuous flow of emulsions was initiated in the system causing them to enter the interaction chamber after 2.6 min. At this point, the HIFU machine delivered tune bursts with repetition rates of 800 msec, a pulse repetition frequency of 1.3 Hz, and a duty cycle of 50% at different pressures of 0.8, 1.14, 1.97, 2.5, 3.0, 3.4 and 4.4 MPa. The linear transducer was then used to image the emulsions and capture the bubbles as they traveled from the
interaction chamber to the waste container. Using the maximum flow rate of 50 ml/min the entire system was flushed for 1 min before introducing the next fresh emulsion sample.

### 2.3.2.2.1 Flow Temperature Profile

As was emphasized throughout this report, the temperature of emulsions is one of the important variables in these experiments. The external water bath was used to adjust the temperature of the emulsions as they traveled in the interaction chamber and before they arrived at the focus of the HIFU. However, since the emulsions were constantly flowing in the system and did not sit in the interaction chamber long enough to equilibrate with the temperature of the water bath, there was a difference in the actual temperature of the emulsions and the temperature of the water bath. Hence, it was desirable to estimate the actual temperature of the emulsions for different water bath temperatures and flow rates. Therefore the experimental setup was temporarily modified to capture the emulsions as soon as they left the interaction chamber to measure their temperature using a laser thermometer. This temperature profile provided a better estimate of the actual temperature of emulsions given the flow rate and water bath temperature.

### 2.3.2.2.2 Water and PFHB as Control Cases

Fowlkes et al. argued that although yet not completely clear, there are several potential explanations for acoustic vaporization of emulsions such as
acoustic cavitation, acoustic heating, shape oscillations during acoustic irradiation, and hydrodynamic cavitation. More detailed explanations of each of these physical phenomena and their relations to ADV are discussed in appendix A.2.

Although rejected by Fowlkes et al. as a possible underlying reason for ADV, it has been shown that high negative pressure of ultrasound waves during acoustic cavitation can generate enough shear force to vaporize pure water in room temperature and produce air gas bubbles [5]. These bubbles travel through the imaging phantom and can be incorrectly detected as microbubbles produced as a result of emulsion vaporization. To ensure that the detected bubbles are due to the emulsion vaporization and not water cavitation, a control experiment was used to determine the range of ultrasound pressures and emulsion temperatures for water and PFHB.

PFHB and PFOB emulsions are relatively stable emulsions with high boiling points of 89°C and 120°C, respectively. Although PFOB would be a better choice as the control material, PFOB was not accessible at the time of these experiments, requiring PFHB emulsions to be used as the second control.

2.4 Data Analysis

Using the linear array US transducer, microbubbles were detected as they were passed through the flow chamber. As shown in figure (17), the horizontal bright lines were the reflection from the tube's upper and lower walls when there was only PFC emulsions in the tube. Once droplets were vaporized into
microbubbles, their impedance was changed and therefore, their echogenicity was increased. This increase was manifested in the form of an increase in pixel intensity values of images and was dependent on the amount and size of gas bubbles produced due to ADV. Therefore, an image processing toolbox from MATLAB was used to develop a script that measured the average echo amplitude (AEA) for the region of interest (ROI) within each frame.

The first image of each series before application of HIFU was used as the baseline background image and all other frames were subtracted from this image before calculating the mean echo amplitude. Therefore in the absence of vaporization, the resulting image was almost black and the AEA was almost zero. As shown in equation (12),

$$\text{AEA} = \frac{1}{M \times N} \sum_{i=1}^{M} \sum_{j=1}^{N} A(i, j)$$

$A(i, j)$ is the signal intensity at pixel $(i, j)$ [14].
Chapter III: Results and Conclusion

3.1 Characterization of PFC Droplets

The boiling points of liquid PFC emulsions were determined as explained in the experimental design section. It has been shown that the boiling point of PFC emulsions is 2 times higher than pure PFC liquid [25]. This is because the emulsification process of liquid PFC reduces the nucleation sites within the solution and therefore solution enters the superheated state before it boils [13]. This was confirmed in this study since a mixture of PFP emulsions boiled at 55°C while its pure form had a boiling point of 24°C, whereas PFHB solution did not boil even at 100°C, given the boiling point of its pure liquid form is 89°C. The emulsion samples with IONP had about the same boiling point as the emulsions without IONP.

Unfortunately PFP emulsions were depleted before the Zetasizer could be used to characterize their size distribution. Regardless, the expected size distribution for this emulsion sample was around 150-200 nm.

3.2 Parameters Optimization

Using the basic experimental setup discussed in 2.3.2.1, the HIFU parameters were optimized for acoustic vaporization of PFHB and PFP emulsions. As shown in table (2), HIFU had 18 predefined settings with fixed repetition cycles, repetition rates, and duty cycles.
After a series of experiments with different settings, mode 25 with repetition cycle of 800 msec and a duty cycle of 50% was selected as the optimum setting for the purpose of this experiment. The HIFU was applied for 0.5 seconds and the power ranged from 0.8 MPa to 4.4 MPa.

Table 2: Preset values for parameters of the HIFU

<table>
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<tr>
<th>Sonication Preset</th>
<th>ON pulse [msec]</th>
<th>OFF pulse [msec]</th>
<th>Duty Cycle [%]</th>
<th>Repetition Cycle [msec]</th>
<th>Repetition Rate [Hz]</th>
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<td>7350</td>
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For the earlier experiments, the ACUSON Antares US machine was used to detect MBs in the imaging phantom. The first imaging phantom, which was made out of PDMS, used the 9 MHz linear array transducer (VFX9-4) to detect the MBs. Due to a high attenuation coefficient of PDMS material, a high US
power (>30%, MI>2) and a low frequency setting was required to obtain any meaningful images. High gain (>40dB) and low frequency, on the other hand, produced low quality images with high signal-to-noise ratios. In addition, the high power used in this setup could destruct the microbubbles even before they were imaged. The second set of experiments used a 6% agar phantom. Agar gel had a very low US attenuation coefficient and its acoustic impedance was very close to water. Therefore both VFX9-4 and VFX13-5 (13 MHz linear array) transducers could be used to detect the microbubbles. The lower gain (17dB) and lower power (>12% MI=0.2) required for this setup generated higher quality images with less background noise.

For the final set of experiments, the ACUSON Sequoia ultrasound system was used. As mentioned earlier, this machine was equipped with CSP technology and was capable of generating high quality images to detect the microbubbles with a very high accuracy. The imaging phantom, a thin walled polyester tube with a low attenuation coefficient, allowed for a low power (-13%, MI<0.19) and high frequency (14MHz) setting.

3.3 Initial Proof of Concept

The initial sample was prepared by diluting PFP emulsions with saline solution at a 1:10 ratio. Figure (9) shows an ultrasound image of the silicon tube filled with this PFP sample at room temperature, which was taken before the application of US energy by the VFX9-4 transducer at 3.8 MHz central frequency and under THI mode with 9dB gain and 12% power (MI~0.2). This sample was
used to optimize the US imaging parameters and provided a base line of pixel intensities for future comparisons.

After the HIFU parameters were optimized, different HIFU intensities were delivered to fresh samples of PFP emulsions ranging from 0.8MPa to 3.4MPa and the resulting images for each pressure were compared. Despite the saturation and shadowing artifacts presented in these images, comparing figures (10) and (11), one can visually confirm a higher average pixel intensity values for 1.97 MPa than 1.14 MPa. These artifacts were due to high bubble densities that were produced at higher ultrasound powers which generated a large bubble concentration in the tube.

As previously mentioned, the temperature of emulsions could also affect the threshold and rate of droplet vaporization. Figure (12) and (13) compare the bubble production inside the chamber due to the 1.14 MPa US pulse at room temperature of 24°C versus emulsions at higher temperatures of 28°C. However, similar to the case of higher pressure, as discussed above, image saturation and shadowing artifacts present in these images made any attempt to quantify these differences ineffective.

Since the emulsification process decreases the nucleation sites within pure liquid PFC, emulsions may enter superheated state. Ultrasound can be used to generate shear forces within these emulsions to boil them. However, the reverse could also be true, indicating that liquid droplets in their superheated state might spontaneously start to vaporize into microbubbles without the application of ultrasound energy. This phenomenon, as mentioned in chapter 1
and 2, is the main source of failure for some of the current therapeutic applications. Therefore, to ensure that higher temperature emulsions did not spontaneously vaporize without the application of HIFU, the chamber was imaged after heating the emulsions and before applying the US energy. As shown in figure (14), emulsions did not vaporize before the application of HIFU.

Heating the emulsions to a high enough temperature could however, vaporize the solution without application of US pulses. Figure (15) shows PFP emulsions with a boiling point of about 45°C spontaneously started boiling at around 55°C before HIFU was applied to the tube. One could argue that these bubbles were due to the air bubbles and not vaporization of emulsions. Also the vacuum created by the syringe reduced the local ambient pressure and could boil the emulsions as they were transferred to the silicon tube. However, 55°C at room atmospheric pressure was not a high enough temperature to boil the water in the solution and create air bubbles. In addition, boiling of emulsions at around 55°C in the test tube was visually confirmed before they were carefully transferred into the silicon tube.

3.4 Droplet Vaporization and Microbubble Detection

Once the US imaging parameters as well as the HIFU settings were optimized, a more complete experimental setup, discussed in chapter 2, were designed to produce more quantitative data. In the proceeding sections, images in each figure present a single frame out of 150 frame video clips recorded during 1 minute flow of solution in the system after they were exposed to HIFU and left
the interaction chamber. These images were selected as a part of a collection of other frames that demonstrated the efficacy of the experimental method described in this report.

### 3.4.1 Emulsion Temperature Profile

As the temperature of emulsions was one of the control variables in these experiments, characterizing its dynamics under different circumstances was crucial. Figure (16) shows the effect of the water bath temperature on the temperature of emulsions at different flow rates. A range between 24°C to 50°C was selected because this is a clinically feasible temperature range for emulsions to achieve *in vivo*. The higher the flow rate, the larger the difference between the temperatures of emulsions and hot water bath as they reached the HIFU focus point. Also, increasing the temperatures of the water bath created a larger gradient between the temperature of emulsions at 24°C and the temperature of the water bath, which explains the larger gap between the lines at this region. Performing these experiments provided a better understanding of the thermal properties of the HIFU interaction chamber and emulsions themselves to better optimize the flow rate and the dimensions of the interaction chamber for future experiments.

### 3.4.2 Water and PFHB as Control

As discussed before, water and PFHB were used as control solutions to establish a range of temperatures and ultrasound powers to ensure that the
produced bubbles are due to the PFP emulsions rather than cavitation of water or air bubbles trapped in the system. This range was then applied to PFP droplets with and without IONP to prove our hypothesis.

During the initial experiments, a range of acoustic pressures between 0.8 to 4.4 MPa was established. Since the boiling point of PFHB and PFP are 89°C and 29°C respectively, the temperature range for these experiments was determined to be between room temperature (24°C) to 70°C for water and PFHB, and room temperature to 40°C for PFP emulsions to ensure that it reached its superheated point. Figures (17) to (19) show the effect of temperature at 24°C, 35°C, and 40°C on cavitation of water at 4.4 MPa, respectively. As shown in the enclosed region of interest, vaporization of water did not occur. This, however, was expected because neither the water temperature nor the ultrasound power was high enough to induce acoustic or thermal cavitation.

Figure (20) on the other hand, shows the same flow of water after its temperature was raised to 70°C and a 2.5 MPa US pulse was applied. In this case, since the temperature of water is closer to the boiling point, application of HIFU at 2.5 MPa induced cavitation and produced some bubbles, which are shown on the figure by circular markers.

Increasing the power up to 4.4 MPa in the case of water, temperatures less than 70°C did not produce any bubbles. This, however, was consistent with published data in literature which reported up to 9 MPa US pressure using a 1 MHz transducer at room temperature [20]. It has been also shown that US frequency and the ADV threshold for PFC emulsion are inversely proportional.
[13]. This, however, implies that using our 0.22 MHz HIFU system, the energy required to vaporize pure water at room temperature might even be higher than 9 MPa.

In addition to water, PFHB emulsions, which also have a high boiling point, were used as the second control solution to establish a range of temperatures and ultrasound pressures for ADV. Figures (21) to (23) indicated the effect of temperature on PFHB emulsions under constant ultrasound pressure. As shown in these figures, application of up to 4.4 MPa US pressure did not vaporize the PFHB emulsions at lower temperatures. At higher temperatures, however, the required pressure to vaporize PFHB emulsions decreased. Figure (24) shows the PFHB gas bubbles that were produced at 65°C under ultrasound pulses of 3.0 MPa. This result was almost similar to the result that was observed for the case of pure water.

3.4.3 Effect of Ultrasound Pressure on ADV

In a real clinical application, a lower temperature range between 24°C to 45°C is more desirable. Therefore, further investigation on the effect of temperature, US power, and encapsulated IONPs was conducted using PFP emulsions which have a boiling point of 29°C. To study the effect of increase in ultrasound pressure on the rate of droplet vaporization, the temperature of the water bath and the flow rate were kept constant, while the US intensity was increased from 0.8 MPa to 4.4 MPa. Figures (25) to (28) clearly demonstrate higher concentration of bubbles at higher US pressures. Another interesting
result, which was observed in these video clips, was the production of smaller bubbles (microbubbles) compared to the bigger ones at higher pressure amplitudes. Smaller bubbles formed white, cloud-like clusters that flowed up the tube with a flow rate closer to that of emulsions at 5 ml/min. Whereas the bigger bubbles were observed as a series of bright single spots on the screen, which traveled in the tube with relatively high speed. This difference in the rise time was due to the effect of gravity on the bigger bubbles compared to the smaller ones.

Although the higher intensity in the later images could be visually confirmed, a MATLAB script, described in section 2.4, was used to better quantify these differences at different ultrasound pressures. Average echo amplitude of the region of interest was calculated over 70 frames and was plotted versus the pressure amplitude corresponding to those 70 frames. This plot, figure (29), shows a higher bubble density within the ROIs for frames corresponding to higher US pressure.

### 3.4.4 The Effect of Temperature on ADV

In a similar fashion to section 3.4.3, the PFP emulsions were used to quantify the effect of the temperature on the rate of droplet vaporization. The ultrasound power and flow rate were kept constant, while the temperature of emulsions swept over a broad range from room temperature at 24°C to about 10°C over the PFP boiling point of 40°C. The bubble density was recorded for three temperatures in this range. As shown in figures (30) and (33), an increase in the temperature of emulsions increased the concentration of microbubbles and
therefore produced brighter pixels with higher intensity values. In addition to the rate of droplet vaporization, figure (34) shows the effect of the temperature on the threshold of pressure required for phase conversion of the liquid droplets to gas bubbles. As shown in this figure, the minimum pressure required to vaporize the droplets at 24°C was 3.4 MPa which was greater than the 3.0 MPa ADV threshold for emulsions at higher temperatures.

In addition to higher pressure, as mentioned in the previous section, higher temperature also produces smaller bubbles that follow bigger ones in cloud-like clusters traveling with a slower flow rate. Figure (35) shows an example of these clusters after 13 second post HIFU.

The higher density of bubbles at higher temperatures produced images with higher pixel intensities (white pixels) compared to the background, which was visually confirmed. However, a more quantitative description was obtained by using the custom MATLAB data analysis algorithm which plots the average intensity values (AEA) of 70 frames versus the temperature of emulsions corresponding to those frames. Figure (29) shows the increase in AEA value for higher temperatures at three temperature points.

3.4.5 Effect of IONPs on ADV

In addition to temperature, the effect of IONPs encapsulated inside the PFC emulsions is another control variable for this project. The presence of IONP inside the emulsions provides additional nucleation sites, which in turn may decrease the US power required to vaporize the PFC droplets. The objective of
this part of the experiment was to determine the ADV threshold of PFC emulsions without encapsulating IONP and compare that with US power required to vaporize IONP loaded droplets.

To investigate this effect, all the other factors such as temperature, flow rate, HIFU setting, and US imaging system parameters were kept constant. Initially a sample of diluted PFHB emulsion (1:20 ratio) entered the system at a water bath temperature of 65°C where US energies ranging from 1.97 MPa to 4.4 MPa were applied. As shown in figure (35) and (36), US energy at 2.5 MPa did not induce acoustic vaporization of PFHB emulsions. Only when the US pressure was increased to 3.0 MPa, very scattered single microbubbles were produced and detected using the imaging system. Although not shown here, the induced vaporization of PFHB emulsions did not exceed several single microbubbles even up to 4.4 MPa. This can be seen on the graph of figure (39), which quantified the bubble concentration inside the tube in relative units.

Keeping the setup the same, a ISCO pump was used to flush the system from any remaining PFHB emulsions and possible microbubbles before applying the IONP loaded PFHB emulsions into the system. The same experiment was performed as above with PFHB emulsions with IONP. Figure (39) shows the effect of IONP at reducing the ADV threshold from 3.0 MPa to 2.5 MPa. This could even be lower that 2.5; however, values below 2.5 MPa were not included in these experiments. Figure (39), on the other hand, demonstrates the effect of higher pressure on the rate of vaporization of emulsions with and without IONPs.
In addition, unlike the plain PFHB liquid droplets, IONP loaded emulsions produced cloud-like clusters of microbubbles that flowed in the system with a much slower velocity than larger bubbles and generated higher brightness and intensity values in the images. As shown in the graph of figure (39), the mean echo amplitude values calculated for IONP loaded droplets were significantly higher for different pressure values than those of PFHB emulsions alone.

3.4.6 Overall Result

As discussed earlier in chapter I, the ultimate objective of this project was to generate a graph that shows the effect of temperature and presence of IONPs inside the emulsions on ADV threshold and rate of droplet vaporization. Figure (40), shows a graph of AEA versus the power of the US for different temperatures of PFP emulsions. In order to superimpose the graph of figure (39) on this plot it was necessary that a same material (PFP emulsions with and without IONP) be used. However, since the data for IONP was gathered from PFHB emulsions, superimposing these images did not provide any meaningful conclusion.

3.5 Conclusion

It has been observed that the PFDDA coated IONPs have a higher suspension ratio in brominated PFC solutions such as PFHB and PFOB. The initial proof of concept experiment has indicated that a combination of longer
repetition cycles and duty cycles were required to induce vaporization in PFP emulsions. Using the predefined sonication setting 25, this was achieved at repetition cycle of 400 msec and a duty cycle of 50%. As it was discussed earlier in this chapter, these images did not provide any quantifiable data.

Using water and PFHB emulsions as a control, a range of temperatures and US pressures were established to ensure that the microbubbles produced in later experiments were indeed due to emulsion vaporization and not to boiling of water in the system. Since both pure water and PFHB have high boiling points, no bubbles were produced up to 70°C and high US pressure of 3.0 MPa. Therefore, vaporization of diluted PFP emulsions, which were heated up to 40°C, was indeed due to droplet vaporization.

Although not applicable in clinical settings, PFP emulsions were diluted with saline at 1:20 ratio to ensure enough concentration of emulsions to induce droplet vaporization. The results of these later experiments showed the effect of higher temperature and pressure on the rate of acoustic vaporization of droplets. Emulsions with lower boiling points were used to demonstrate that while keeping the US power constant, higher temperatures produced more microbubbles.

The next series of experiments showed that the presence of IONP inside the emulsions could decrease the acoustic threshold of droplet vaporization. Similar to the effect of temperature, bubbles produced at lower acoustic power for emulsions that encapsulated IONP present external nucleation sites. In addition, figure (39) clearly shows the effect of IONP on increasing the rate and concentration of produced microbubbles in the system.
Another interesting result observed in these experiments was the production of cloud-like clusters of microbubbles in higher temperatures and higher pressures. These microbubble clusters are more desirable than bigger bubbles.

As shown in the previous figures, in addition to microbubbles that flowed in the system as cloud-like clusters, a series of bigger sized bubbles were produced. This was especially the case for lower powers and temperatures. This however, might cause problems during in vivo clinical applications of this technology since these bubbles could block the vessels and stop blood flow.

3.6 Limitations

There were few limitations that required special attention as they could be used to explain some of the unexpected results of these experiments and could be improved in the future studies.

As mentioned in chapter 1, when US waves pass through materials with acoustic impedance greater than water, the attenuation of acoustic power occurs. Also, scattering and reflection of US waves with external material, which in this case are produced by hundreds of transducers in the HIFU system, will cause a shift in HIFU focal point. Both of these factors were true in these experiments as the interaction between the US waves and the wall of the water bath could reduce the pressure at the focus as well as shift the focal point in the center of the HIFU. The HIFU system therefore, required a special hydrophone to locate its
focal point and determine its exact acoustic pressure at the focus. However, this specialized hydrophone for the HIFU was not available and therefore alternative means such as change in the temperature of water inside the transducer housing was used to detect the location of its focus inside the water bath.

Placing a tip of a thermocouple inside the water of the transducer housing, the temperature rise was observed for different locations as the HIFU was delivering 500 Watts of electric power to the focus point for 2 minutes. This power and duration of HIFU intensity increased the temperature of the focus point by almost 8°C from 24°C room temperature to 32°C. Temperature change outside of the focus where the power of the HIFU drops by 90% was minimal.

In addition to estimate the HIFU acoustic pressure at its focal point, equation (10) was used. Not only did this equation estimate the actual pressure delivered at the focus, it did not take into account the scattering and attenuation of the wave due to the water bath in the center of the HIFU, both of which could change the actual value of the pressure. However, especially at higher pressure, the distortion of the US beams due to the water bath were neglected.

Another limiting factor for this studied application was the post processing algorithms used by clinical ultrasound scanners to generate the final image. Therefore after manipulation of data through different stages of linear or non-linear image processing algorithms, the final image did not necessarily represent the raw data in any linear fashion. Although the mean echo amplitude of the captured images used the average intensity values, the effect of non-linear
correlation between the pixel intensities and the rate of microbubble production was clearly shown in the graph of pressure versus temperature.

PFHB was used to demonstrate the effect of IONP on reducing the acoustic threshold of droplet vaporization and had a high boiling point close to 90°C. However, this temperature is not applicable in clinical settings and has a less accurate result since water could cavitate close to that temperature and US pressure. However since at the time of these experiments, IONP loaded PFC emulsions were not available, PFHB emulsions with and without IONP was used.

In order to generate a more accurate representation of the effect of temperature and IONP on the vaporization threshold and rate of microbubble production, more data points from similar material was required. This will be proposed as the future direction of this project to obtain a more accurate representation of the effect of these factors on ADV.

3.7 Future Direction:

Once vaporization of the droplets and detection of microbubbles \textit{in vitro} and then consequently \textit{in vivo} is accomplished, the ultimate goal of this project is to use emulsions for drug delivery purposes. We envision that using drug loaded emulsions with encapsulated IONP and moderate pressure generated by classical US systems can be used to collapse the formed microbubbles to deliver drug, DNA, and any other nano-systems.

In addition, the IONP encapsulated in the emulsions can be used to serve two different purposes. The supermagnetic properties of IONP make them
accessible to an external magnet, which can be used to remotely guide the emulsions through an external force to the target site. Also, IONP can efficiently convert the magnetic energy of an external alternative current magnetic field (ACMF) to heat, and therefore can be used to heat the emulsions to a superheated state \textit{in vivo}. ACMF uses radio frequency power and surface coil in a safe frequency and amplitude to generate localized hyperthermia.

As an alternative application for this technology, detecting the vaporization process itself can also be studied. In brief, when a phased-array transducer receives a signal pulse of sound from tissue, the time-of-flight from the point that the sound was generated to each transducer element is defined. This data can then be used to generate a high amplitude sound, such as in HIFU applications, to coagulate a small tissue volume. However, currently it has not been a non-invasive way to generate sound \textit{in vivo}. Therefore, the vaporization of emulsion can be used as a platform to act as the source of this sound.

Lastly, it is worth mentioning that the findings of these studies may not represent droplet behavior in blood. It has been shown \textit{in vitro} that the ADV threshold increases when blood is the host medium. Also, the dilution ratio of 1:20, which was used in this case, is not applicable for \textit{in vivo} studies. Therefore, the next step of this project could use blood as the transport media and increase the dilution ratio to 1:100 or 1:1000 liquid PFC droplets to saline.
3.8 Figures

Figure 3: Transducer housing of the HIFU machine

Figure 4: Computer unit used to control the output of the HIFU
**Figure 5:** Water-bath placed in the center of the HIFU transducer

**Figure 6:** Interaction chamber between the HIFU waves and emulsions
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Figure 10: PFP emulsions in silicon tube at the room temperature 22°C, HIFU at 1.14MPa

Figure 11: PFP emulsions in silicon tube at 22°C, HIFU at 1.97MPa
Figure 12: PFP emulsions in the silicon tube at 22°C, HIFU at 1.14MPa

Figure 13: PFP emulsions in the silicon tube at 28°C, HIFU at 1.14MPa
Figure 14: PFP emulsions in the silicon tube at 50°C before applying the HIFU

Figure 15: Spontaneous boiling of emulsions at 55°C
Figure 16: Temperature profile for emulsions under 5ml/min and 2ml/min flow rate
Figure 17: Flow of water in the tube at the room temperature (24°C) US pressure of 4.4MPa.

Figure 18: Flow of water in the imaging tube at 35°C US pressure of 4.4MPa, Region inside the tube is labeled as ROI
Figure 19: Flow of water in the tube at 40°C HIFU at 4.4MPa. Region inside the tube is labeled as ROI.

Figure 20: Flow of water in the tube at 70°C HIFU at 2.5MPa, bubbles are shown by circular markers.
**Figure 21:** Flow of PFHB in the tube at 24°C HIFU at 4.4MPa, region inside the tube is labeled as ROI

**Figure 22:** Flow of PFHB in the tube at 35°C HIFU at 4.4MPa. Region inside the tube is labeled as ROI
**Figure 23:** Flow of PFHB in the tube at 35°C HIFU at 4.4MPa. Region inside the tube is labeled as ROI.

**Figure 24:** Flow of PFHB in the tube at 65°C HIFU at 3.0MPa. The moving MBs are marked by a circular geometry.
**Figure 25:** Flow of PFP in the tube at 35°C HIFU at 0.8MPa. The moving MBs are marked by a circular geometry.

**Figure 26:** Flow of PFP in the tube at 35°C HIFU at 1.14MPa. The moving MBs are marked by a circular geometry.
Figure 27: Flow of PFP in the tube at 35°C HIFU at 2.5MPa

Figure 28: Flow of PFP in the tube at 65°C HIFU at 4.4MPa.
Figure 29: Mean echo amplitude of vaporized PFP emulsions at 35°C
Figure 30: Flow of PFHB in the tube at 24\(^\circ\)c HIFU at 3.4MPa. The moving MBs are marked by a circular geometry.

Figure 31: Flow of PFHB in the tube at 35\(^\circ\)c HIFU at 3.0MPa.
Figure 32: Flow of PFHB in the tube at 40°C HIFU at 3.0MPa

Figure 33: Flow of PFHB in the tube at 40°C HIFU at 3.0MPa
Figure 34: Mean echo amplitude of vaporized PFP emulsions at 4.4MPa
Figure 35: Flow of PFHB in the tube at 65°C HIFU at 2.5MPa

Figure 36: Flow of IONP loaded PFHB emulsions in the tube at 65°C HIFU at 2.5MPa
**Figure 37:** Flow PFHB emulsions in the tube at 65°C after it was hit by HIFU at 3.0MPa.

**Figure 38:** Flow of IONP loaded PFHB emulsions in the tube at 65°C HIFU at 3.0MPa.
Figure 39: Effect of IONP encapsulated in PFHB emulsion vaporization rate and threshold at 65°C

Figure 40: Overall effect of pressure and temperature on the rate of ADV.
Appendix

A.1 Cadence Contrast Pulse Sequencing Technology

A new technology is now available on some clinical imaging systems that recognizes and processes the unique nonlinear fundamental and higher order harmonic signals that are generated by ultrasound contrast agents. New pulse sequence technology is ideally designed for contrast agents. The agent-to-tissue specificity of contrast imaging is significantly increased and therefore provides improved performance over current systems.

It is now understood that by varying the phase and amplitude of multiple pulse interactions with a contrast agent, the agent response is unique and can be efficiently separated from the tissue signals. Therefore, this new technology simultaneously processes received signals from multiple transmitted pulses of varying phase modulation and varying amplitude modulation. This unique response is termed nonlinear, as it arises as a result of the bubble’s nonlinear expansion and contraction with the ultrasound pulse. This response is also termed fundamental, as the bubble’s strongest returned response is at the same frequency as the transmitted pulse. Therefore, the precise control of pulse amplitude and phase allows the detection of strong \textit{nonlinear fundamental} energy exclusively from the contrast agent.

\textbf{Phase Modulation}

Changing phase: A bubble’s nonlinear expansion and contraction is sensitive to the initial phase as shown in Figure 41 and 42 in these examples, the
received pulses on the far right from two excitation pulses of opposite phase help identify the bubble from tissue. The recorded signals are not simply inverted copies of each other, unlike that of tissue.

**Amplitude Modulation**

Changing amplitude: In further describing the interaction of contrast agent bubbles with ultrasound, consider a pressure pulse and the positive, compression section of the pulse which compresses a bubble. When the bubble is compressed, a fixed amount of sound is scattered and reflected back to the transducer. Next, consider a second pressure pulse that is twice as large as the first with twice the amplitude. In this case the bubble is compressed more than during the smaller, first pulse. Keeping in mind that a bubble expands much more easily than it contracts, in a nonlinear fashion, the section of the second pulse does not just simply return half as much energy as the first one. This non-linear oscillation of bubbles is the bases for amplitude modulation.

With Cadence CPS technology, the fundamental frequency is being transmitted and then the returned nonlinear fundamental frequency as well as higher order harmonics is being detected from the wobbling bubbles. This technology then combines multiple received pulses to extract the strong nonlinear signals. However, spectral filters that separate signals in the frequency domain are not effective for separating linearly scattered fundamental tissue signals from nonlinearly generated fundamental bubble signals, as both signal types are in the same fundamental frequency band. Instead, separation is achieved by proprietary combinations of multiple pulses with Cadence CPS
technology. Proper amplitude and phase combinations support effective tissue signal rejection and bubble signal extraction all within the same fundamental frequency band.

**Figure 41:** Phase modulation used in CPS technology, a non-linear response of bubble to pulse
Figure 42: Phase modulation used in CPS technology, a non-linear response of bubble to pulse2

A.2 Sequoia Cadence CPS Technology

Sequoia platform used in these experiments uses this cadence contrast pulse sequencing (CPS) technology. Although Cadence CPS technology encompasses the design of many different pulse sequences for different imaging characteristics, one example of a Cadence CPS technology implementation and pulse break down is discussed.

As shown in figure 43, initially a half-amplitude, positive pulse is transmitted and the processed received signal includes a linear fundamental tissue and nonlinear fundamental contrast agent, and nonlinear harmonic contrast agent components. As the transmit power is very low, the nonlinear tissue harmonic component of the received signal is very low and can be
suppressed with processing inherent in the Sequoia system’s Coherent Image former. The linear tissue response and the nonlinear fundamental response carry the same polarity as the transmitted pulse, while the nonlinear harmonic response carries its own polarity.

Next, a full-amplitude, negative polarity, 180 degree, pulse is transmitted and the processed received signal includes similar significant components as the first received signal, which were linear fundamental tissue, nonlinear fundamental contrast agent, and nonlinear harmonic contrast agent components. The linear tissue response and the nonlinear fundamental response, again, carry the same polarity of the transmitted pulse. However, due to the bubble’s nonlinear behavior, the nonlinear fundamental contrast response from this pulse exhibits a higher amplitude than the nonlinear fundamental contrast response from the first half-amplitude pulse. In addition, the nonlinear harmonic response carries its own polarity.

Lastly, another half-amplitude, positive and zero degree pulse is transmitted and in this case the processed received signal includes similar significant components as the first two received signals. The linear tissue response and the nonlinear fundamental response carry the same polarity as the transmitted pulse while the nonlinear harmonic response carries its own polarity. The amplitude of the non-linear fundamental contrast response is similar to the first received pulse. When the received composite sequence of signals is summed, the tissue components from the half-amplitude, positive polarity pulses 1 and 3 equal the full amplitude, negative polarity pulse 2 and therefore cancel
out. The contrast agent’s nonlinear fundamental signals add to form a significant, strong signal.

Cadence CPS technology has the unique ability to combine the nonlinear fundamental and higher order harmonic contrast signals to form a highly specific and sensitive contrast agent display. By utilizing this sequencing strategy, the Cadence CPS technology can effectively separate tissue signal from contrast agent signal, or can combine them together.

Cadence CPS technology is an attractive technology for higher frequency imaging, where acquiring useful harmonic frequencies would be beyond the bandwidth of today’s state-of-the-art transducer technologies. High frequency imaging offers spatial resolution on the order of several hundred microns, which may be applied to areas such as the human breast, thyroid, testicle, or carotid arteries. The emerging field of small animal imaging for use in genomics, pharmaceutical, or other research studies can also benefit from improved resolution at high frequencies with excellent agent-to-tissue specificity.
A.3 Physical Mechanism for Acoustic Droplet Vaporization:

The phase conversion of liquid emulsions into MBs due to application of acoustic pressure is called acoustic droplet vaporization (ADV). Up to date there is no accurate explanation for the physical mechanism behind this phenomenon. Some of the possible mechanisms for ADV are (a) acoustic and hydrodynamic cavitation which could potentially nucleate the evaporation of the superheated droplet; and (b) deformation of the droplet which could lead to rupturing of the shell, including redistribution of lipid on the droplet’s surface [6,7], and/or (c) a non-uniform Laplace pressure on the sphere.
In general cavitation is defined as a process in which micro gas bubbles are created as a consequence of a reduction in local pressure in the liquid. Acoustic cavitation is one of the four mechanisms which were under investigation to explain how ADV might work. Experiments have shown that by lowering the dissolved gas content of liquid media by 37%, the threshold for cavitation increased by 10%. However, the measured acoustic pressure threshold for ADV was approximately two standard deviations away from the estimated value [33]. Based on the 2 standard deviation difference, transient cavitation is probably not the mechanism for ADV. In addition, the cavitation threshold is a direct function of frequency whereas; experiments have shown that ADV and frequency of US pulse are inversely related [5,11]. This could be used to justify our conclusion that acoustic cavitation is not an underlying mechanism for ADV.

Effects of repetitive pulses could be used to test if acoustic heating was the basic mechanism for ADV. When working on ADV of emulsions flowing through a tube, there are two ways in which change in the repetitive pulses might manifest itself by changing one of the two; (a) the fraction of droplets which will be vaporized per unit time, and (b) the pressure threshold that has to be overcome to start ADV.

During the analysis of shift in the pressure threshold for ADV due to the change in pulse repetition frequency (PRF), it was observed that over two-orders of magnitude change in PRF the threshold for ADV changed only by 11%, which is not significantly different [33]. Other parameters which could contribute to heating include burst length, ambient temperature, and ambient viscosity.
Current results of the PRF study do not support heating as a mechanism for initiation of ADV. However, more experiments would be required to definitely determine that heating is not involved.

In addition, droplet shape oscillations causing a non-uniform Laplace pressure were found to be 15% or less of the droplet diameter. They could be observed at the beginning and at the end of the acoustic irradiation.

Spot-like onset of vaporization was observed during the dipole-type motion of droplets. This onset was solely seen on the axis of oscillation close to a pole of the droplet. The nucleation did not coincide with the occurrence of the shape oscillations. Based on these observations and the high Reynolds number during ADV ($4-5\times10^5$), it is concluded that the mechanism of vaporization might be based on hydrodynamic effects.
References:


