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Acoustic Intensity Measurement System: Application in Localized Drug Delivery

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ACOUSTIC INTENSITY MEASUREMENT SYSTEM: APPLICATION IN
LOCALIZED DRUG DELIVERY

A Thesis

Presented to

The Faculty of the Department of Electrical Engineering
San Jose State University

In Partial Fulfillment

of the Requirements for the Degree

Masters of Science

by

Natalie Phipps

May 2010

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The Designated Thesis Committee Approves the Thesis Titled
ACOUSTIC INTENSITY MEASUREMENT SYSTEM: APPLICATION IN
LOCALIZED DRUG DELIVERY

by
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APPROVED FOR THE DEPARTMENT OF ELECTRICAL ENGINEERING
SAN JOSE STATE UNIVERSITY

May 2010

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ABSTRACT

ACOUSTIC INTENSITY MEASUREMENT SYSTEM: APPLICATION IN
LOCALIZED DRUG DELIVERY

by Natalie Phipps

Ultrasound is used for many applications in diagnostics and therapy. New developments are being made specifically for the use of therapeutic ultrasound for enhancement of localized drug delivery. The long term goal of this study is to find ultrasound pulsing sequences that will allow for controlled mass diffusion from hydrogel drug reservoirs. Developing this new method requires the calibration and characterization of therapy transducers using power and acoustic intensity measurements. Mechanical index, spatial peak pulse average intensity, and spatial temporal pulse average intensity calculations were made using hydrophone measurements set-up in a tank measurement system. These were compared to safety limits defined by the Food and Drug Administration. With these calculated safety values, preliminary tissue mimicking phantom measurements were made to verify the possible efficacy of the new method. The membrane of the microcapsule bulged under continuous sonication from the transducer. Further investigation is needed to identify the mechanisms responsible for this effect.

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1. INTRODUCTION

The process of getting new medical devices or procedures into clinical practice is lengthy and multi-phased. A procedure or device can only be used in clinical trials after equipment calibration, tissue mimicking construct (phantom) tests, in-vitro tests, and in-vivo tests. The purpose of these development phases is to ensure the safety and efficacy of the new device or procedure. As detailed in the highlighted portions of Fig. 1, this thesis will discuss ultrasound pulse sequence development, material development, and preliminary phantom experiments for a new ultrasound based drug delivery method.

Fig. 1. Initial phases of interdisciplinary drug delivery study

Material development includes formulating phantoms and drug reservoirs (microcapsules). Ultrasound pulse sequence development includes measuring electrical input power applied to the therapy transducer and acoustic intensity emitted from the therapy transducer. Optical measurements will include observing changes in phantoms and microcapsules that are undergoing ultrasound sonication with a transmission light video microscope.

1.1 Breast Cancer

The American Cancer Society estimates that 40,170 women died in the United States due to breast cancer in 2009 [1], making it the second most common cause of cancer death in women [2]. Between 1991 and 2006, the death rate among women has steadily declined from 32.7 to 23.4 deaths per 100,000 [1]; this is a relative change of 28.3%. While this trend can be partially attributed to a successful campaign for breast cancer screening, new strategies are still needed for prevention, detection, and treatment to reduce overall mortality rates.

1.1.1 Current Treatments

Current treatment methods for breast cancer include surgery, systemic therapies, and localized therapies. Often large tumors are surgically removed and then therapies are used to treat the residual cancer cells [3]. Some of these therapy methods include: radiation therapy, chemotherapy, hormone therapy, and monoclonal antibody therapy [1]. In systemic chemotherapy drugs are injected intravenously to kill rapidly dividing cancer

cells. During the journey to the cancer cells, these drugs may bind to other rapidly dividing cells or become metabolized [4]. This causes healthy bone marrow, hair, skin, and intestinal cells to be destroyed. Some common side effects include nausea, hair loss, mouth sores and throat sores [5]. A trade-off has to be made in chemotherapy treatment between delivering an effective drug dose and preventing toxicity.

Targeting the cancer cells locally with optimal quantities of drug can mitigate the side effects seen in systemic chemotherapy. For example, a method of local drug delivery is with using thermosensitive liposomes that encapsulate anti-cancer agents [6]. These constructs undergo permeability changes when exposed to heat sources such as microwaves and infrared lasers [6]. Changes in the membrane permeability allow the release of the drug payload with lower systemic toxicity. Unfortunately, these constructs rely on the chaotic tumor vasculature for transport and are too small (nanometers in diameter) to supply an effective dose of drug over the entire cancer cell lifecycle.

1.1.2 Basics of the Tumor Microenvironment

Systemic chemotherapy is unable to reach all cells in a cancerous tumor because the tumor microenvironment contains leaky vasculature, lack of functional lymphatics, and lower vasculature density [3]. Blood flow in the tumor varies widely among the four different regions of a tumor: necrotic tissue region, semi-necrotic tissue region, stabilized microcirculation region and rapid cell growth region [4]. These necrotic masses form because of nutrient deprivation in their respective regions. Other methods of treatment

must be performed in coordination with chemotherapy because of the tumor's vascular arrangement.

1.2 Ultrasound

Ultrasound describes a subset of mechanical waves in the frequency range of 0.02–2,000 MHz [7], which is above the audible frequency range for humans [8]. Clinical ultrasound operates in the range of 1–15 MHz [7]. While ultrasound waves can propagate in four different operating modes [7], only longitudinal waves are relevant in tissue imaging and therapy. Table 1 summarizes the properties of ultrasound.

Longitudinal waves occur when the particles of a medium vibrate about their resting location in the direction parallel to wave propagation. The wave contains zones of alternating compression and rarefaction. Compression occurs when the particles of the medium vibrate opposite to the direction of wave propagation. This creates a region in the medium that is denser with sound pressure waves than at ambient pressure.

Rarefaction occurs when the particles vibrate in the direction of wave propagation. This creates a region in the medium that is less dense with sound pressure waves than at ambient pressure [9]. Fig. 2 shows that the wavelength, λ , represents one full cycle of compression and rarefaction. Wavelength is related to f , the frequency, and c , the velocity of wave propagation, which ranges from 1,450–1,600 m/s depending on the tissue type [10]. The equation for calculating wavelength is

$$\lambda = \frac{c}{f}. \quad (1)$$

As ultrasound waves propagate through a medium, beam intensity is reduced by reflection, scattering and absorption. The energy from the beam is absorbed by particles of the medium that become heated. The rate of energy absorption or tissue attenuation varies from 0.3–0.8 dB/cm/MHz depending on the tissue type [7]. This highlights that attenuation is a function of distance traveled and wave frequency.

TABLE 1. Summary of Ultrasound Properties

Property Type	Value
Frequency Range	0.02–2,000 MHz, most common from 1–15 MHz for medical devices [7]
Velocity in Soft Tissue	1,450–1,600 m/s, depending on the tissue type [7]
Source Device	Transducer, piezoelectric elements convert between electrical and mechanical energy [7]
Wave Type	Mechanical, requires a medium to propagate [9]
Operating Modes	Longitudinal, transverse, surface, and plate [9]

Fig. 2. Longitudinal ultrasound waves

1.2.1 Diagnostic versus Therapeutic Ultrasound

Diagnostic and therapeutic ultrasound applications have different electrical input waveforms characteristics, which results in different acoustic intensity outputs.

Diagnostic ultrasound has a maximum pulse duration of 1 μ s [7], while therapeutic ultrasound can operate with continuous wave for several minutes. Acoustic intensity is the energy flow per second per unit of cross sectional area [9] and is an indicator of possible bio-effects. Lower temporal average acoustic intensity waves are used in diagnostic ultrasound (0.017–0.720 W/cm²) [10], while higher acoustic intensity waves are used in therapy ultrasound (0.1–10,000 W/cm²) [11]. The applications of ultrasound therapy range from physiotherapy to high intensity focused ultrasound in thermal ablation of prostate cancer cells [3]. High ultrasound intensities can result in primarily three mechanisms for causing bio-effects: heat generation, acoustic cavitation and acoustic radiation force [3].

1.2.2 Ultrasound in Drug Delivery

Bio-effect mechanisms caused by high intensity ultrasound have generated interest among those who are researching new methods for localized therapy. If these mechanisms can be safely and effectively employed to alter tissue or drug agent permeability, it could result in an increased absorption or delivery level of anti-cancer agents in tumors.

1.3 Long Term Goals and Scope of Thesis

The long term goal in this multi-phased study (see Fig. 1) is to find a combination of drug reservoir characteristics and a transducer pulse sequence that will allow time controlled drug elution with the primary consideration to patient safety.

The scope of this thesis is to build a system that will calibrate and characterize an acoustic transducer that will be used for mass transport from a microcapsule construct. In particular it will include transducer power measurements, acoustic beam characterization, and preliminary phantom testing under a microscope.

2. LITERATURE REVIEW

In the following discussion the development and challenges of ultrasound in different drug delivery methods will be discussed. This literature review is broken into five sections: basics of enhanced drug delivery, a brief history of ultrasound in medicine, ultrasound mechanisms and transducer characterization, and applications and challenges of ultrasound in cancer treatments.

2.1 Basics of Enhanced Drug Delivery

Enhanced drug delivery methods are developed in order to change biological properties of tissue or drug vehicle [4]. The purpose of changing biological properties of tissue is to reduce transport barriers that prevent drug uptake. Some current technologies used to reduce these barriers are hyperthermia, radiofrequency ablation, electroporation, magnetic fields, and acoustic cavitation with ultrasound [12]. An example of hyperthermia would be the Krol *et al.* study that incubated ex-vivo cells at temperatures from 37–41° C. This resulted in the reducing of the number of viable cells and increasing the available fraction of drug [3]. Drug vehicles are used to position the drug in the vicinity of the cancerous region so that drug will primarily diffuse into the cancerous region. An example of changing properties of the delivery vehicle is liposomes that release drug when exposed to heat [6]. Ultrasound is a more recent method for enhancing drug delivery. It is an attractive approach to drug enhancement because it is non-invasive, inexpensive, and can access deep tissues.

In any drug delivery approach it is important to understand the method by which the chemical is delivered to the cancerous body. In order for chemotherapy to be effective, the correct therapeutic agent and dose must be chosen to treat the region [4]. In a blood born treatment, the drug must cross from blood vessel, through the vessel wall, and into the interstitium (space between cells) [4]. Along this path the drug binds randomly to healthy cells or becomes metabolized [4]. Heterogeneous movement of molecules in the tumor is determined by the blood flow rate and the number, length, diameter, and geometric arrangement of blood vessels [4]. Systemic chemotherapy is not sufficient for delivering drug to cancerous cells in this environment.

2.2 A Brief History of Ultrasound in Medicine

Ultrasound is known to be a safe and valuable diagnostic imaging method. Diagnostic ultrasound became popular primarily because it posed a lesser health risk than X-ray [13]. Before ultrasound was used as a diagnostic imaging modality, it was used for physiotherapy heat treatments. High intensity therapeutic ultrasound was slow to develop due to uncertainty with respect to safety and there being no consistent way compare human exposure to laboratory studies [14]. This led to the development of testing and labeling standards by American Institute of Ultrasound in Medicine (AIUM) and National Electrical Manufacturers Association (NEMA), which are now maintained by the FDA [14]. These standards define appropriate safety parameters such as ultrasound power, spatial peak temporal average intensity (I_{spta}), spatial peak pulse average intensity (I_{sppa}), and mechanical index (MI).

Many ultrasound therapies have been developed since these standards were established. Treatments developed included lithotripsy for the treatment of kidney stones and thrombolysis for the treatment of blood clots [14]. Therapeutic ultrasound specifically for drug delivery is currently used in sonophoresis, a technique of sonicating skin to enhance transdermal drug diffusion [12]. Extensive research is being done to apply this method to drug delivery to solid tumors [15]. These will be discussed in detail in section 2.4.

2.3 Ultrasound Mechanisms and Transducer Characterization

There are three well known risks to high intensity focused ultrasound: hyperthermia, acoustic radiation force and acoustic cavitation. Hyperthermia is when the rise in temperature causes proteins to denaturize and necrose [12]. Acoustic radiation force occurs when momentum is transferred from the emitting wave to the tissue to elicit tissue motion [3]. These effects have not been well studied with respect to temporary or permanent tissue responses [14]. Acoustic cavitation occurs when oscillating pressure changes due to the ultrasound wave causes oscillating bubbles to form in liquid media like blood vessels or cysts, leading to the disruption of cells, blood vessels and tissue structures [12]. The presence of these mechanisms is determined by spatial and temporal acoustic power and intensity characteristics of the ultrasound transducer pulse sequence. The highest level of acoustic intensity for diagnostic imaging is 0.72 W/cm^2 [10] and ranges from $100\text{--}10,000 \text{ W/cm}^2$ for therapy applications [11]. In order to effectively and

safely use a transducer, the location and area of the highest focal intensity must be known.

Transducers are normally characterized with an acoustic radiation force balance or an acoustic raster scan system. A radiation force balance works by operating the transducer directly over a target, *e.g.* cone, rubber, brush, and oil filled bag [11]. The change in vertical position of the target due to the radiating force of the beam is sensed by mechanical (weighing balance) or magnetic (electromagnetic coil) means and converted into a force [8]. An acoustic raster scan system translates an acoustically sensitive device called a hydrophone to different positions within the transducer beam. These measurements are then converted from electrical power to acoustic power by the hydrophone sensitivity constant at that frequency. The advantage of this system over the acoustic radiation force balance system is that the entire beam can be spatially characterized.

2.4 Applications and Challenges of Ultrasound in Cancer Treatments

Applications of high intensity focused ultrasound include thermal ablation of cancer cells and localized mediated drug delivery. Thermal ablation uses high intensity focused ultrasound (HIFU) and is currently in clinical use for certain types of cancers like prostate cancer [12]. Thermal ablation is performed by exposing tissue to several minutes of high power ultrasound. The focused beam allows tissue in the near and far field of the beam to remain unharmed, while directly targeting the cancerous tissue at the focus [12]. This

procedure can be difficult to perform because cancerous tissues are often very close to healthy tissues and appropriate monitoring mechanisms need to be in place.

Ultrasound mediated drug delivery is another application of ultrasound in cancer treatments. A pulsing scheme set with a low duty cycle allows the tissue to cool by diffusion and convection during the off part of the cycle [3]. This still enables the high peak pulse to cause cavitation effects needed to create temporary holes in the membrane. Cavitation is difficult to induce since there is a lack of dissolved gases in the blood stream [12]. When cavitation does occur, the timing and effect is hard to monitor or control.

One solution is to add small gas bubbles or ultrasound contrast agents (UCAs) into the blood stream to provide a nucleus in which a cavitation bubble can oscillate. This allows cavitation to occur at lower acoustic intensities resulting in fewer complications due to the heating of healthy tissue. These UCAs cannot diffuse through the vessel wall because they are protected by a lipid or protein outer layer. Direct applications of this effect are seen in sonoporation where direct and transient opening of the cell can be modulated for gene delivery [12]. This solution was also found to create very strong and damaging reactions to tissue cells when the UCA are destroyed.

The long term goal of this research is to encapsulate drug and UCAs into reservoirs (microcapsules) in order to protect healthy cells from complications of acoustic cavitation. This would contain the destructive nature of the UCA while keeping the membrane of the microcapsule intact up to the acoustic threshold. Microcapsules are made from polymer based shells and range from micrometers to a few millimeters. It has

been previously noted that microencapsulation allows better biodistribution of the drug and has shown promising results with thermosensitive liposomes [6]. Even with the successes of thermosensitive liposomes, new methods still need to be developed to enhance localized mediated drug delivery.

3. METHODS

3.1 Electrical Input Power Measurement

Traditional diagnostic transducers are powered with high voltage (100 V) spikes to generate short pulses for imaging. For therapeutics, the input voltage applied can range from short pulses to continuous wave. A continuous wave is normally used for radiation force, whereas short pulses are used to engage cavitation effects. Being able to modulate the input voltage in terms of the voltage, duty cycle and overall power is important for generating useful pulses for drug delivery design. A simple function generator combined with an RF power amplifier can be used to power the transducer. Calibration tests as well as electrical power measurements were performed.

The power spectrum of a pulsed waveform is difficult to measure accurately when the signal has a pulse repetition period (PRP) in tens of milliseconds, a pulse duration (PD) in microseconds, and a period (T) in tens of nanoseconds (see Fig. 3). This is because testing parameters, *e.g.* sweep time and sample rate, will drastically change the measured or calculated power.

For power measurements, the data was sampled in the time domain with an oscilloscope at 50 MHz for the length of the pulse repetition period (10 ms) and processed in the time and frequency domains to calculate power. This method provided more processing options when compared to measuring with a spectrum analyzer.

Fig. 3. Time representation of a pulsing scheme

3.1.1 Fundamental Concepts for Electrical Input Power Measurement

3.1.1.1 Data sampling

The number of samples (N) needed to produce a power measurement without distortion due to aliasing is described by [16]

$$N = f_c * PRP * n * q \quad (2)$$

where f_c is the operating frequency of the transducer, n is the number of bursts captured, and q is the number of samples taken per oscillation period. Although the Nyquist rate defines the minimum value of q to be two, a higher value should be chosen to minimize

distortion. If the value of q is too high, it will be difficult to store and process the data in order to calculate power values.

3.1.1.2 Windowing

Windowing is a finite impulse response (FIR) filtering method that involves modifying sampled data to produce a desired Fourier magnitude response. This is done by truncating or tapering samples in the time domain. It is described by the equation [16]

$$x_w(n) = x(n) * w(n) \quad (3)$$

where n is the index value, $x(n)$ is the sampled data, $w(n)$ is the applied window and $x_w(n)$ is the filtered data. The purpose of windowing is to correct for frequency spectral leakage defined as power that is concentrated at frequencies other than the original signal spectrum due to biases in finite length data processing methods. These spectral leaks appear as side lobes on the spectrum. A rectangular window truncates the samples, which results in high side lobe levels and a narrow main lobe at the center frequency. Most other windows, *e.g.* Blackman window, gradually taper the samples to zero in the window period, which results in low side lobe levels and a wide main lobe at the center frequency. With any window chosen, there is a trade-off between narrow main lobe around the center frequency (for high frequency resolution) and low side lobes (for less spectral leakage). The equation for a rectangular window [16] is

$$w(n) = 1 \quad (4)$$

where n is an index value. The equation for Blackman window [16] is

$$w(n) = 0.42 - 0.50 * \cos\left(\frac{2\pi n}{N-1}\right) + 0.08 * \cos\left(\frac{4\pi n}{N-1}\right) \quad (5)$$

where n is an index value and N is the total number of samples.

3.1.1.3 Power with discrete Fourier transform

Once the appropriate window filter is applied to the time domain data, the discrete Fourier transform (DFT) is used to convert the data from time domain, $x_w(n)$, to frequency domain, $X(f)$ by [16]

$$X(f) = \sum_{n=1}^N x_w(n) e^{(-i2\pi fn)}. \quad (6)$$

Power is relative to the maximum value of the DFT squared by [16]

$$P(f) = |X(f)|^2 / R \quad (7)$$

where $X(f)$ is the value of frequency domain representation of the input voltage, and R is the value of system loading. Since the relative peaks in the power spectrum are easier to view in log scale, (7) is converted into dBm by

$$P(f)_{\text{dBm}} = 10 \log[P(f)/10^{-3}]. \quad (8)$$

3.1.2 Experimental Setup for Electrical Input Power Measurement

Electrical input power measurements are necessary because it can be used to control the acoustic output of the transducer. This is done by generating an electrical pulse with the combination of waveform generator and RF power amplifier. The output voltage of the power amplifier was attenuated with a 10x probe to ensure that the signal did not exceed the voltage rating of the oscilloscope ($5V_{\text{rms}}$ with 50Ω load setting). The oscilloscope samples the waveform and sends the data via IEEE-488 General Purpose Interface Bus

(GPIB) to the computer for post-processing. The system requirements for each component are detailed in Table 2. The setup for the electrical input power measuring system is shown in Fig. 4.

TABLE 2. Equipment Specifications for RF Power Measurement

Device	Specifications
Arbitrary Waveform Generator Tabor Electronics WW2571A	Remotely programmable Multiple signal types (CW, burst, modulated) Sine waves generated to 100 MHz
RF Power Amplifier Electronic Navigation Ind. 240L	Flat frequency response over 20–10 MHz 50dB gain
Oscilloscope Agilent DSO6034A	Remotely programmable 2 GSa/s sampling rate 50 Ω load option 4 channels
PC Software	Labview and Matlab

Fig. 4. Setup for electrical input power measurement system

3.1.3 Measurements and Summary

Data sets were acquired over a pulse repetition period of 10 ms for 1–10 burst counts (sine wave frequency: 2.25 MHz, duty cycle range: 0.0044–0.044%) at 50 mV input from the function generator. Other data sets were acquired by varying the input voltage between 20 mV and 100 mV keeping the burst counts constant at five. With (6)–(8) power values were calculated.

This calibration study allowed verification of the RF power amplifier device performance over a range of input voltages and burst counts. If transducer device efficiencies are available, correlation of electrical input power to expected acoustic power can be easily made. These correlation measurements can be used as a guide to choosing a pulsing scheme relevant to drug delivery applications.

3.2 Acoustic Intensity Measurement System for a Therapy Transducer

The intent of ultrasound in therapy is to cause bio-effects that will improve the health of the patient. The pressure and intensity of the ultrasound beam was measured spatially with a hydrophone and compared with accepted mechanical and thermal parameters defined by the Food and Drug Administration (FDA).

3.2.1 Fundamental Concepts for Acoustic Intensity Measurement

A single element focused transducer was measured in the axial and lateral planes that contain the focus. The transducer converts electrical input power into mechanical vibrations or acoustic energy by the piezoelectric effect. The piezoelectric effect is seen

in the transducer when the crystalline elements deform proportional to the strength and polarity of the applied electric field causing mechanical stress on a medium [17]. Any piezoelectric material can create and sense mechanical waves because the effect is reversible.

In Fig. 5, the axial beam is the acoustic variation in the y-z plane and the lateral beam is the acoustic variation in the x-z plane. Focal width, focal depth, ultrasound harmonics, mechanical index, spatial peak pulse average intensity and spatial temporal average intensity were calculated with this data. Definitions of these terms will be discussed next. Table 3 at the end of the section gives a summary of all units used in these terms, unless locally described.

Fig. 5. Lateral and axial beam definition of a single element transducer

3.2.1.1 Ultrasound harmonics

The transducer emits primary harmonics at center frequency f_c along with contaminating harmonics at multiples of the center frequency ($2f_c$, $3f_c$, etc.) in the acoustic pulse [18].

The amplitude and bandwidth of the second harmonic ($2f_c$) is of most interest because it contains the most power. In preliminary tissue mimicking construct (phantom) experiments, the transducer will emit pulses into a tissue-mimicking medium with ultrasound contrast agents (gas filled micro-bubbles). Testing in this medium will cause intrinsic harmonics of the ultrasound contrast agent and distortion harmonics from wave propagation in the tissue medium [18].

3.2.1.2 Center frequency

The center frequency is the operating frequency of the transducer and is used to calculate the hydrophone sensitivity. While this value is given by the supplier, there are always slight manufacturing variations that will affect the value. The equation is

$$f_c = \frac{(f_1 + f_2)}{2} \quad (9)$$

where f_1 and f_2 are the frequencies on either side of the spectral peak at 3 dB below maximum spectral power.

3.2.1.3 Pulse repetition frequency

The pulse repetition frequency is set by the waveform generator, but still needs to be measured and verified since it is a value used in the calculation of the safety parameters.

The pulse repetition frequency (PRF) is the inverse of PRP shown in Fig. 3. If the time between sequential pulses t_1 and t_2 is short, it will result in high PRF and the average temporal acoustic intensity output of the transducer will be high. It is described as

$$PRF = \frac{1}{t_2 - t_1} \quad (10)$$

where t_1 is the time of the first burst cycle and t_2 is time at which the next burst cycle occurs.

3.2.1.4 Hydrophone voltage, acoustic pressure, and focal area

A hydrophone is a device that can measure mechanical pressure waves in liquids. Like transducers, hydrophones convert acoustic pressure waves into voltage waves by the piezoelectric effect. Unlike transducers, hydrophones are able to sense acoustic excitation over a broad range of frequencies and at high spatial resolution. Hydrophones are very expensive and sensitive instruments that must be handled with great caution and care. The quality of the instrument degrades with any contact from a hard surface or even prolonged exposure to water.

The sensitivity curves of the hydrophone and preamplifier must be used to convert the voltage output of the hydrophone into acoustic pressure. These curves are normally provided by the manufacturer. Voltage can be converted into acoustic pressure by [19]

$$M_l(f) = G_a(f) * M_c(f) * \left(\frac{C_h}{C_h + C_a} \right) \quad (11)$$

where $G_a(f)$ and C_a are the preamplifier gain and capacitance, and $M_c(f)$ and C_h are the hydrophone EOC sensitivity and capacitance.

After the voltage output of the hydrophone has been converted into acoustic pressure, compression and rarefaction pressure values can be measured. Peak rarefactional pressures are achieved when the region in a medium is the least

concentrated with sound pressure waves because particle oscillations are opposite the direction of propagation (see Fig. 2). It is also the value used to plot the axial and lateral planes of the transducer in order to calculate focal depth and focal width respectively. It is defined as the absolute value of the most negative pressure from the hydrophone output described by [10]

$$p_r = \frac{|\min(v(t))|}{M_1(f_c)} \quad (12)$$

where $v(t)$ is the voltage seen by the hydrophone and $M_1(f_c)$ is the hydrophone sensitivity conversion factor at the center frequency.

3.2.1.5 Pulse intensity integral and pulse duration

Intensity is the power per unit area. It is defined as [10]

$$PII = \frac{\int_{t_1}^{t_2} v^2(t) dt}{\rho c * M_1^2(f_c)} \quad (13)$$

where $v(t)$ is the hydrophone voltage, M_1 is the hydrophone sensitivity at the center frequency, t_1 and t_2 represents the time duration of interest, and ρc is the specific acoustic impedance.

Specific acoustic impedance is the level of resistance experienced by the propagating wave. It is a value used to calculate all of the safety parameters. For a longitudinal wave, the impedance is described by [10]

$$\rho c = \rho * \left[\frac{Y(1 - \sigma)}{\rho(1 + \sigma)(1 - 2\sigma)} \right]^{1/2} \quad (14)$$

where ρ is the material density, c is the velocity of sound, Y is Young's modulus, and σ is Poisson's ratio [9]. Young's modulus is a measure of stiffness in an elastic material and Poisson's ratio is the ratio of transverse strain over axial strain.

At room temperature, the density and longitudinal velocities of water are 1000 kg/m³ and 1480 m/s respectively [9]. This results in $\rho c = 1.48 \text{ MPa} \cdot \text{s/m}$ at 293 K. This value will be rounded to 1.50 MPa · s/m for simplicity and used in later calculation.

A conversion factor to account for tissue calculations and attenuation is applied to the PII with the equation [10]

$$\text{PII}_{\text{derated}} = \text{PII} * e^{-0.115 * \alpha * f_c * z} \quad (15)$$

where the factor 0.115 is the conversion between decibels (log base 10) to Neper (natural log), α is tissue attenuation, and z is the distance from the transducer to depth of interest. The accepted value for tissue attenuation is 0.3 dB/cm/MHz.

The pulse duration is the amount of time that the ultrasound pulse is considered on. It is calculated from the pulse intensity integral. The equation is [10]

$$\text{PD} = 1.25 * (t_2 - t_1) \quad (16)$$

where t_1 is the time when the amplitude is 10% below peak PII and t_2 is 90% below peak PII.

3.2.1.6 Intensity values (I_{spta} and I_{sppa})

Spatial peak temporal average intensity (I_{spta}) is the maximum intensity occurring over the pulse repetition period. It indicates the thermal deposition by ultrasound [10]. Spatial peak pulse average intensity (I_{sppa}) is the maximum intensity in the beam averaged over the pulse duration [10]. The formulas used to calculate these values are as follows [10]:

$$I_{spta} = PII_{derated} * PRF \quad (17)$$

$$I_{sppa} = \frac{PII_{derated}}{PD} \quad (18)$$

where $PII_{derated}$ is the derated pulse intensity integral, PRF is the pulse repetition frequency and PD is the pulse duration.

3.2.1.7 Mechanical index

The mechanical index is a measure of the probable negative bio-effects experienced from the applied ultrasound wave. All devices currently approved by the FDA must have a mechanical index lower than 1.9 [10]. This index can be computed as [10]

$$MI = \frac{Pr_{derated}}{\sqrt{f_c}} \quad (19)$$

where $p_{r,derated}$ is the derated peak rarefactional pressure at the location of the maximum peak intensity integral and f_c is the center frequency in megahertz.

TABLE 3. Units for Measured Parameters

Value (units)		
f_c, f_1, f_2 (MHz)	p_r (MPa)	α (dB/cm/MHz)
t, t_1, t_2 (seconds)	v (volts)	z (cm)
PRF (Hz)	ρ_c (MPa $\cdot$ s / m)	PD (seconds)
M_l, M_c (V / Pa)	Y (Pa)	I_{spta} (W / cm ²)
G_a (unitless)	σ (unitless)	I_{sppa} (W / cm ²)
C_h, C_a (pF)	PII, PII _{derated} (μ J / cm ²)	MI (unitless)

3.2.2 Accepted Values of Safety Parameters

Table 4 describes some of the index levels allowed for safe use in diagnostic ultrasound.

Therapeutic ultrasound does not have maximum levels that are determined by the FDA.

The purpose of the maximum levels seen in Table 4 is to prevent bio-effects in the corresponding tissue. The therapies in Table 5 are specifically trying to create bio-effects for therapy purposes. These are not values that are regulated by the FDA but rather suggested values accepted by the industry to gauge acoustic intensity. Both guidelines will be used to determine safe levels of exposure.

TABLE 4. Suggested FDA Acoustic Output Exposure Levels - Diagnostic

Use	I_{SPTA} (W/cm ²)	I_{SPPA} (W/cm ²)	MI (unitless)
Peripheral Vessel [10]	0.720	190	1.9
Cardiac [10]	0.430	190	1.9
Fetal Imaging & Other [10]	0.094	190	1.9
Ophthalmic [10]	0.017	28	0.23

TABLE 5. Common Acoustic Output Exposure Levels - Therapeutic

Use	I_{SPTA} (W/cm ²)
Physiotherapy [11]	0.1-1
Lithotripsy [11]	Very low
Haemostasis [11]	100-5,000
HIFU [11]	400-10,000
Drug Delivery [15][12]	Very low-10,000

3.2.3 Experimental Setup for Acoustic Intensity Measurement System

The experimental setup consisted of a 2.25 MHz focused therapy transducer submerged in a tank that was lined with acoustic rubber and filled with clean, de-ionized, degassed water. A three-axis motion controller was attached to the tank and had an arm that positioned a needle type hydrophone into the tank. A PC with a software controller (Labview) was able to position the hydrophone with 0.005 mm step precision. The voltage of the hydrophone was measured over lateral and longitudinal planes of the focus. The hydrophone signal was increased with a preamplifier and the signal was recorded with an oscilloscope and stored to the controller PC. Fig. 6 shows the tank setup. Fig. 7 shows the complete system setup.

3.2.3.1 Software controller

The Labview controller was comprised of individual blocks called VIs that issued commands to the instruments. These commands were sent via IEEE-488 General Purpose Interface Bus (GPIB) and used equipment-specific protocol; however, commands were loosely based on Standard Commands for Protocol Instruments (SCPI).

The waveform generator, oscilloscope, and three-axis motion controller all had its own controller VI that was integrated into one top-level controller VI. Basic VIs were

Fig. 6. Tank setup for acoustic measuring system

Fig. 7. Acoustic measuring system setup

supplied by the manufacturers of the equipment, but modifications and coordination was necessary to implement a properly functioning scanning system.

The waveform generator VI from the manufacturer was designed to output continuous wave standard waveforms such as sine, square wave, ramp, etc. A custom VI was created since this application required pulsed waveforms of varying duty cycles. This custom VI allowed the user to generate a pulsed waveform with a defined duty cycle and to select the appropriate voltage output.

The oscilloscope VI from the manufacturer was designed to capture a waveform of 1000 samples after the device had undergone an auto-scale. The sampling rate was insufficient for this application since a large number of samples were needed to capture high frequency bursts at a low frequency pulse repetition frequency (see Fig. 3). The only way to capture the needed number of samples was to capture the maximum number of samples allowed by the device (two million samples when the time duration was 0.01 s). Then this data set was decimated to 50,000 samples for 8 μ s of data, a sample number determined by (2) to balance the problems of under-sampling and unmanageable data processing times.

The default VI for the three-axis motion controller contained all of the commands that the device could perform. The commands that were not necessary for this application were removed. The user was left with the option of opening the port, inputting instrument commands for motion, and closing the port. A Matlab program was written in order to generate commands to move the instrument automatically. The user could customize length of the sides of the scanning plane, time delay between

movements, and number of movements within that scanning area. The scanning area was 9 mm x 9 mm for the axial scan and 1.4 mm x 1.4 mm for the lateral scan. This was selected after course scanning was performed to determine the approximate beam area.

The top level VI used included the waveform generator, oscilloscope and three-axis motion controller. Fig. 8 shows how the Labview controller was integrated into the experimental setup.

Fig. 8. Overview of the Labview controller in acoustic measuring system

The VI performed the following commands: (a) send configuration data to the waveform generator and powered on the voltage output, (b) send relative position data changes to the motion controller, and (c) request electrical voltage data from the

oscilloscope. To scan the transducer voltage over many different positions, (b) and (c) were repeated until the entire requested area was scanned.

3.2.3.2 Transducer

The transducer used in this experiment is a 2.25 MHz immersion high power therapy transducer with a cylindrical focus at 1.25 in. (3.175 cm) (Valpey Fisher Inc. IL0208HP-SF=1.25). An immersion transducer emits ultrasound wave only in liquids and solids since air is not a good enough conductor of sound. The exact choice of frequency is arbitrary for now but future studies need to be done to choose the right frequency for the drug delivery application.

3.2.3.3 Hydrophone and preamplifier

The trade-off in choosing a hydrophone is between sensitivity (large active element) and spatial resolution (narrow acceptance angle). The hydrophone and preamplifier chosen for this experiment are the HNP-0400 and the AH-2010 (20 dB gain) from Onda Corp. This needle type hydrophone has a nominal sensitivity of 50 nV/Pa over the 1–20 MHz frequency range and an acceptance angle of 60° both of which are best for these measurements.

3.2.3.4 Three-axis motion controller

A three-axis motion controller is used to move the hydrophone over the axial and lateral plane of the transducer in the water tank. This instrument is made by Velmex Inc. and is

controlled with custom Labview program that was built especially for this purpose. A custom arm was also built in order to lower the hydrophone into the tank for measurements. The range of motion can be adjusted on this device by moving the safety stops but within a maximum limit of a 125 in.³ (2048 cm³). The transducer beam width for this focused transducer is in the order of a few millimeters, which makes the 5 μ m step precision of this instrument essential.

3.2.3.5 Water tank

A custom tank was designed and made in the SJSU machine shop. The dimensions of the tank are 9 in. x 12 in. (22.9 cm x 30.5 cm), which allowed for full range of motion by the three-axis motion controller without the risk of hitting the hydrophone against the tank wall. This tank has a threaded hole in the side to allow the transducer to be screwed into the side while remaining water tight. The tank is fixed with clamps to maintain a consistent position throughout the tests. It allows the tank to be removed for cleaning. The tank is lined with a quarter inch acoustic rubber (McMaster Carr Corp.) to prevent any acoustic reflections.

The effectiveness of the measurement is greatly impacted by the quality of the testing medium. Any impurities in the water can become reflected and change the power measurement greatly. The water was degassed by boiling distilled or DI water for 20 minutes and was stored in air tight containers in the refrigerator. This creates a vacuum in the container until the water is used. The water was brought to 20° C, so that the speed

of sound was consistent over experiments on different days. Guidelines for making degassed water were taken from [20].

3.2.4 Measurements Taken and Summary

In each experiment it was necessary to perform a manual search of the transducer focus. Since the focal length is just a few millimeters, there is no automated way to line up the transducer using entirely mechanical means. These experiments were run with a burst cycle of five and a peak-to-peak input voltage from the arbitrary waveform generator at 50 mV. This generated 5 mW electrical power input to the transducer (see Fig. 13 and Fig. 17).

To measure the lateral plane (see Fig. 5), the hydrophone was translated over nine points in the x direction and nine points in the z direction (total 81 points) with 0.2 mm step precision in each direction. The hydrophone voltage was captured and stored onto the controller PC.

To measure the axial plane (see Fig. 5), the hydrophone was translated over the 20 points with 1.0 mm step precision in the y direction and 12 points with 0.5 mm step precision in the z direction (total 240 points). The hydrophone voltage was captured and stored onto the controller PC.

With this data, measurements of critical safety metrics like I_{spta} , I_{sppa} and MI were calculated and the lateral plane was plotted to view the focal width. With this data, measurements of the axial plane were plotted to view the focal depth. With this system any relevant pulsing scheme in drug delivery can be tested to determine if it is in

accordance with FDA safety levels or within the range of other therapeutic methods.

This acoustic system will also provide information about the beam profile so that an investigator can determine if the chosen transducer is appropriate for the therapeutic task at hand.

3.3 Preliminary Experiments with Tissue Mimicking Phantom

The effect of an ultrasound pulse from the transducer was tested with acoustically sensitive microcapsules suspended in tissue mimicking constructs (phantoms). The materials and methods for this experiment were developed between the Electrical Engineering and General Engineering Departments. This collaborative effort will continue in future development of this drug delivery method. The effect of the ultrasound pulse on the microcapsules and phantoms were observed using a transmission light video microscope where images were analyzed frame by frame to record any changes.

3.3.1 Material Development for Phantom Testing

The materials in this experiment were essential for determining an appropriate pulsing scheme. Without a consistent, stable material there can be no repeatability in the results when finding an appropriate pulsing scheme.

3.3.1.1 Ultrasound phantoms

Phantoms are tissue mimicking constructs that are used to develop new devices, calibrate equipment, and train medical professionals. The phantom must be spatially, thermally and temporally uniform to provide useful results in therapy development. Phantoms can be formulated to mimic magnetic, electrical, optical, thermal and mechanical properties of a particular type of tissue for one or more imaging modalities. This experiment required an optically clear phantom so that changes in the microcapsules under sonication could be seen under a microscope.

The constructs for initial phantom testing were made with a mixture of 10% transparent gelatin and DI water in a petri dish mold (3 mm height by 30 mm in diameter). The gelatin solution was heated until it became clear at approximately 60° C. Mold release was sprayed in the petri dish prior to pouring the gelatin mixture so that the phantom could be removed and placed in a custom stand-off designed for the experiment. Then the acoustically sensitive microcapsules were separated from their solution and carefully placed in the bottom of the petri dish. Heat was applied to the petri dish using steam to prevent the gelatin from solidifying before the bottom of the dish could be evenly coated with 3 mm of gelatin. Finally, the phantom was covered with plastic to prevent dehydration and placed in a refrigerator to solidify.

3.3.1.2 Acoustically sensitive microcapsules

The following microcapsule method was formulated by Dr. Maryam Mobed-Miremadi who specializes in microencapsulation and has published several papers on this topic.

An acoustically sensitive microcapsule (ASM) is a shell that is sensitive to acoustic pressure and coats and protects material in its interior. ASMs of different sizes (100–2000 μm) were loaded with a mixture of drug-like substance (blue dextran) and ultrasound contrast agents (UCAs 1–10 μm that carry gas). This experiment used commercially available UCAs (Targesons) that were purchased.

The microcapsule membrane is made of Alginate Poly-lysine Alginate, a common encapsulation material [21]. The microcapsule was made by suspending the blue dextran at a specific concentration in medium-to-high viscosity sodium-alginate that is atomized into calcium chloride solution [21]. This is done in an atomizer chamber with coaxial air flow. The size of the droplets was controlled by the flow-rate of the coaxial air, the flow-rate of the sodium-alginate suspension and the radial dimensions of the atomizer. The gelled droplets were coated with poly-lysine during an adsorption step resulting in a hydrogel membrane. Finally, sodium-alginate within the capsule is liquefied and incubated with sodium citrate. On average each microcapsule contained 10–15 Targesons. Newer methods are being developed to increase the density of Targesons in the capsules to engage acoustic effects at potentially lower intensities.

3.3.2 Transport Methods for Phantom Testing

Transport methods are ultrasonic pulsing schemes that will move Targesons to the edge of the microcapsule to potentially facilitate the controlled release of drug substance from the microcapsule.

3.3.2.1 Acoustic radiation force

Acoustic radiation force (ARF) is a force applied to a medium by a sound wave [22]. It is produced due to four physical effects: density changes of propagating waves, spatial variation of energy density in standing acoustic waves, reflection from inclusions or other interfaces, and spatial variation in propagation velocity [22]. An application of ARF is its use in elasticity imaging (displacement of tissue in coordination with ultrasonic imaging to observe mechanical properties) [23]. Other applications include monitoring therapy, molecular imaging, and acoustical tweezers [22]. ARF was used in this experiment to displace ultrasound contrast agents with each microcapsule in the solid medium to push against the membrane of the microcapsule material.

3.3.2.2 Acoustic cavitation

Acoustic cavitation is the occurrence of vapor cavities inside a liquid when its pressure has been lowered below vapor pressure [24]. In a medical ultrasound application, it refers to bubbles induced in tissue by ultrasonic pressure [14]. When high acoustic rarefactional pressure is applied, small cavities are compressed and begin to pulse [25]. Two types of cavitation effects can occur: stable and transient. Stable cavitation is when a bubble forms and grows over multiple cycles of the acoustic intensity. Its effects can cause surface wave activity and microstreaming (currents opposite in direction of the main current motion) [26]. Transient cavitation is when the bubble forms and grows within less than one cycle of the acoustic intensity. The effect of these transient bubbles can cause high pressures and temperatures that can erode solids, initiate chemical

reactions and produce luminescence [26]. The long term goal of this study is to determine if these transient bubbles will allow diffusion of drug through a membrane.

3.3.3 Experimental Setup for Phantom Testing

The experiment consists of an arbitrary waveform generator and power amplifier driving a transducer to image microcapsules under sonication using a transmission light microscope. These microcapsules were suspended in the phantom to simulate a very simplistic tissue environment. Fig. 9 shows the experimental setup with a Nikon Epiphot 200, an inverted transmission light video microscope. In order to capture any effects of the ultrasound on the material, the optical and acoustical focus was aligned. This was done by positioning the light of the microscope in the center of the transducer face. The video software of the microscope allowed images to be captured at 7–8 frames per second. Both a stand-off and support clamp stand was used to stabilize and position the transducer over the microcapsules. In the future, a more robust system for aligning the acoustic focus of the transducer and optical focus of the microscope shall be developed. The 3 mm phantom sample was submerged in 31.75 mm of DI water and the transducer was placed above.

3.3.4 Measurements Performed and Summary

For the preliminary experiments, a continuous wave pulse ($f_c = 2.25$ MHz with input voltage 65 mV peak-to-peak) was used to sonicate the material. The above measurement

setup is the first step toward visualizing a potentially important drug delivery scheme using a combination of ultrasound contrast agents and drug substance in microcapsules.

Fig. 9. Phantom testing setup on transmission light microscope

This experiment allowed for basic visualization of the effect of ultrasound on microcapsules. However, the current limitations of the microscope and the stand-off do not allow clear visualization of the ultrasound contrast agents, easy alignment of the acoustic and optical focus, or high speed video for capturing stable or transient acoustic cavitation effects. A more robust experimental setup will be built in the future along with the use of a biological microscope and high speed camera.

4. RESULTS

4.1 Electrical Input Power Measurement

This calibration study showed RF power amplifier device performance over a range of input voltages and duty cycles (burst counts). From these measurements, device efficiency can be calculated if acoustic power is measured and electrical input power can be correlated to expected acoustic power. These correlation measurements can be used as a guide when choosing any pulsing scheme relevant to drug delivery applications.

4.1.1 Burst Count versus Output Power

Figures 10–13 show the effect of varying the burst count from 1–10 on output power.

This range of burst counts was chosen because it produces an acoustic pressure under 2 MPa, which is the maximum pressure that the hydrophone can be exposed to continuously without damage. The expected results from this experiment also correspond to the maximum Food and Drug Administration (FDA) data limits (see Table 4).

Fig. 10. Time domain output of power amplifier over different burst counts

Fig. 11. Power spectrum over different burst counts with Blackman window

Fig. 12. Power spectrum over different burst counts with rectangular window

Fig. 13. Power output versus burst count

Fig. 10 shows a close up view of the time domain output over different burst counts. It appears that the function generator takes approximately one cycle with a period of 44 ns to transition from on to off; *e.g.* when burst count is three there are actually four peaks visible on the graph. Fig. 11 and 12 show the spectral output over different burst counts. Fig. 11 is less noisy than Fig. 12 as expected due to the Blackman window. The purpose of windowing is to correct for spectral leakage that is caused by processing finite length sequences into the frequency domain. As seen in Fig. 11, as burst count increases, the main lobe centered at 2.25 MHz becomes narrower and higher. In an ideal model, the frequency domain representation of a sine wave is a pulse with

infinite amplitude at the center frequency. In Fig. 13, the power output was converted from decibels to watts by a variation of (8). The power output increases with the burst count when input voltage is 50 mV varies in a quadratic manner by the equation

$$P_o = 0.2035 * BC^2 + 0.1213 * BC + 0.0429. \quad (20)$$

These observations show that a Blackman window is a good windowing method and the system behaves approximately according to (7) over 1–10 burst counts. If the efficiency of the transducer were known, this equation could be used to directly calculate the acoustic power output of the transducer. Efficiency is important because low efficiency devices generate a lot of heat, potentially damaging the device and posing a safety risk to the patient.

4.1.2 Input Voltage versus Power Output

Figures 14-17 show the effect of varying the input voltage from 20–100 mV on output power. The lowest voltage that can be produced by the function generator is 20 mV. The samples were clipped because limitations of the 10x probe for any input voltage above 100 mV.

As seen in the burst count experiment, the time domain output shown in Fig. 14 shows a 44 μ s delay between transitioning the burst from on to off mode. Fig. 15 and Fig. 16 show the spectral power output of the power amplifier over different input voltages from the waveform generator. Fig. 15 is less noisy than Fig. 16 due to the Blackman window. In Fig. 16 the power output was converted from decibels to watts by

a variation of (8). The power output increases with the voltage input (V) when burst count is five by the equation

$$P_o = 0.2248 * V_i^2 + 0.3107 * V_i + 0.8750. \quad (21)$$

If the efficiency of the transducer were known, (20) and (21) could be adapted to directly calculate the acoustic power output of the transducer. Again, efficiency is important because low efficiency devices generate a lot of heat, potentially damaging the device and posing a risk to the patient.

The implications from these two measurements is that the power amplifier behaves according to (7) over a range of input voltages and burst counts. This will allow the electrical power output of the power amplifier to be extrapolated over different input voltages and burst count scenarios required during pulse sequence design for drug delivery without direct measurement or calculation. With acoustic power measurements that can be obtained using a radiation force balance, the transducer device efficiency can be calculated over time to assess device damage due to heating with continuous use. Heating can also cause a shortened device lifespan and quality degradation over time.

Fig. 14. Time domain output of power amplifier over different input voltages

Fig. 15. Power measurement over different voltages with Blackman window

Fig. 16. Power measurement over different voltages with rectangular window

Fig. 17. Power output versus voltage input from waveform generator

4.2 Acoustic Intensity Measurement System for a Therapy Transducer

Acoustic intensity measurements are needed to evaluate the efficacy of relevant pulse sequences and compare their characteristics to accepted safety parameters. Also these measurements are required to measure the efficiency of the device and to line up the acoustic and optical focus for phantom experiments.

4.2.1 Fundamental Concepts for Acoustic Intensity Measurement

4.2.1.1 Ultrasound harmonics

Linear spectral amplitude is plotted to see any contaminating harmonics created by the transducer. Fig. 18 shows the harmonics from the received hydrophone voltage. The first harmonic is at 2.25 MHz. The transducer distortion at the second harmonic ($2f_c = 4.5$ MHz) is a peak value of 70μ with a bandwidth of 500 kHz. The third harmonic ($3f_c = 6.75$ MHz) is 35μ and the bandwidth is indistinguishable. The third harmonic should be at 9 MHz but is dominated by noise. Low powered harmonics are expected due to irregularities in the transducer and by the ultrasound pulse propagating through water.

Fig. 18. Linear spectrum of received hydrophone voltage to show harmonics

4.2.1.2 Center frequency

The center frequency is needed in choose the correct sensitivity parameter in order to convert the hydrophone voltage to into pressure. In Fig. 19, f_1 is 2.1552 MHz and f_2 is 2.5220 MHz. The center frequency of the transducer is at 2.3386 MHz. This is a 3.9% difference from the manufacturer declared frequency value of 2.25 MHz.

Fig. 19. Spectral amplitude calculation of center frequency

4.2.1.3 Pulse repetition frequency

The pulse repetition frequency is parameter that can be controlled by the waveform generator but still needs to be measured and verified. This value will be used in the calculation of spatial peak temporal average intensity. The pulse repetition frequency

(PRF) was found by taking samples over four burst periods. The PRF was found to be 100.3 Hz from Fig. 20. This is a 0.3% error from the expected value of 100 Hz.

Fig. 20. Hydrophone voltage with calculated pulse repetition frequency

4.2.1.4 Hydrophone voltage, acoustic pressure, and focal area

The hydrophone converts transducer spatial pressures into voltage. Acoustic pressure is the hydrophone voltage multiplied by the manufacturer's sensitivity value as in (11). Fig. 21 shows the output from the hydrophone in voltage on the left axis and pressure in megapascal , calculated using the sensitivity factor from the hydrophone, on the right axis. The peak rarefactional pressure is 0.5530 MPa shown at the most negative peak of the waveform at 71.4 μ s.

Fig. 21. Hydrophone voltage output with peak rarefaction pressure

The transducer focal width and depth are measured from the peak rarefactional pressure (see Fig. 21) plotted over the lateral and axial planes. The transducer focal width can be measured using Fig. 22. The peak rarefactional pressure at the focus is found to be 0.5530 MPa with an input voltage of 50 mV and burst count of five. The focal width is shown as 1.1 mm, where width is measured at 6 dB below peak value. The focal width is important as it guides the size of the drug reservoirs used for the drug delivery study. It is also used to align the acoustic and optical focus in the tissue mimicking construct (phantom) experimentation. Without proper alignment, no bio-effects can be seen. The transducer focal depth can be measured using Fig. 23 at 7 mm.

The input voltage is 50 mV peak-to-peak and a burst count of five. The focal depth over the 10% drop is 3 mm. The focal width and depth calculated is consistent with values seen for other high intensity therapy transducers [27].

Fig. 22. Lateral contour of the transducer. Measured with $f_c = 2.25$ MHz, distance from transducer (focus) = 31.75 mm. Resulted in max (p_r) = 0.5530 MPa.

Fig. 23. Axial contour of the transducer. Measured with $f_c = 2.25$ MHz, distance from transducer (focus) = $31.75 \text{ mm} \pm 5 \text{ mm}$. Resulted in $\max(p_r) = 0.5530 \text{ MPa}$.

4.2.1.5 Pulse intensity integral and pulse duration

The pulse intensity integral (PII) is the power per unit area. PII curve is shown in Fig.

24. From this curve, a t_1 is $70.73 \mu\text{s}$ and t_2 is $72.37 \mu\text{s}$. This yields a pulse duration of $2.05 \mu\text{s}$. This is a 6.8% error from the expected value of $2.2 \mu\text{s}$ that was calculated using burst count of five and a center frequency of 2.25 MHz.

Fig. 24. Pulse intensity integral and calculated pulse duration

4.2.1.6 Intensity values (I_{spta} and I_{sppa})

Spatial peak temporal average intensity (I_{spta}) is the maximum intensity occurring over the pulse repetition period. Spatial peak pulse average intensity (I_{sppa}) is the maximum intensity in the beam averaged over the pulse duration [10]. The I_{spta} and I_{sppa} values were calculated with (17) and (18) respectively. Fig. 25 shows the I_{spta} on the left axis and the I_{sppa} on the right axis.

Fig. 25. Intensity curve with I_{spta} and I_{sppa}

Fig. 26 shows peak I_{spta} over axial and lateral dimensions (see Fig. 5). With these plots, an estimation of acoustic power can be made by multiplying the maximum I_{spta} with the 6 dB beam area. The maximum I_{spta} is found to be 1.15 mW/cm^2 . The focal depth and width is as 4.0 mm and 0.8 mm respectively.

Fig. 26. Axial and lateral contours with peak I_{spta} . Measured with $f_c = 2.25$ MHz around the focus of the transducer. Resulted in $\max(I_{\text{spta}}) = 1.15 \text{ mW/cm}^2$.

4.2.1.7 Mechanical index

The mechanical index (MI) is a unitless value that is a measure of possible bio-effects. The peak rarefaction pressure is seen in Fig. 21 and is used to calculate MI with (19). The value is found to be 0.39, which is well below the limit of 1.9 limit defined by the FDA (see Table 4).

4.2.2 Summary of Implications

Using the acoustic intensity measuring system, axial and lateral contour plots were measured for a given therapeutic transducer. From the pulse intensity integral, the following values were calculated from the focus for I_{spta} , I_{sppa} , and MI respectively: 0.0059 W/cm^2 , 28.8 W/cm^2 , 0.39. Using Table 4, these levels are below the values for I_{sppa} , I_{spta} , and MI for diagnostic applications. This method can be used to test these safety parameters for any transducer or pulsing scheme that proves to be useful in a drug delivery scenario.

Tables 6-8 give a summary of the data acquired with electrical input power measurements and acoustic intensity measurements. Table 6 shows how electrical input power is related to input voltage and burst count over a range of values. Only values for 50 mV input voltage over 1–10 burst counts along with 20–100 mV input voltage at 5 burst counts were measured. All other values were estimated using Fig. 13 and Fig. 17. Table 7 relates electrical input power to acoustic power assuming transducer efficiency of 90% as reported by the manufacturer and common with single element transducers [14]. Table 8 relates electrical power to peak values of the safety parameters MI, I_{sppa} , I_{spta}

calculated at the focus. Only data for 5 burst counts at 50 mV was measured. Powers and intensities for other burst counts was calculated based on (20) by changing the time over which the integration was performed to the specific burst count number. The purpose of these tables is to provide a reference for power and intensities measurements for a given pulsing scheme. Further measurements need to be done at other input voltages over various burst counts to obtain a comprehensive list for lookup during experimental studies.

TABLE 6. Electrical Input Power from Power Amplifier (mW)

	Input Voltage from Waveform Generator (mV)									
		20	30	40	50	60	70	80	90	100
Burst Count	1	0.2	0.3	0.3	0.4	0.5	0.7	0.9	1.0	1.1
	2	0.7	0.8	0.9	1.1	1.3	1.5	1.8	2.1	2.3
	3	0.9	1.6	2.0	2.4	3.5	5.4	7.2	8.9	10.5
	4	1.1	2.0	3.5	3.5	5.4	8.1	10.5	14.2	15.5
	5	1.2	2.2	4.1	5.6	8.9	10.9	12.7	17.6	22.6
	6	1.8	4.4	7.2	8.1	10.5	12.5	18.5	20.6	25.0
	7	2.0	5.5	10.5	11.5	12.5	15.4	19.4	22.6	28.5
	8	2.7	6.6	12.5	13.6	18.4	21.0	26.7	27.4	30.6
	9	3.9	8.6	15.4	17.6	22.5	22.3	28.4	31.5	35.6
	10	5.4	10.5	19.2	21.6	25.4	28.4	32.5	35.4	40.1

TABLE 7. Acoustic Power (mW) for 50 mV Input from Waveform Generator

Burst Count	Electrical Input Power (mW)	Average Acoustic Power (mW) using transducer efficiency
3	2.4	2.1
4	3.5	3.0
5	5.6	4.5
6	8.1	7.2
7	11.5	10.2
8	13.6	11.5

TABLE 8. Peak MI, I_{sppa} , I_{spta} for 50 mV Input from Waveform Generator

Burst Count	Electrical Input Power (mW)	MI (unitless)	I_{sppa} (W/cm ²)	I_{spta} (W/cm ²)
3	2.4	0.24	17.8	0.0037
4	3.5	0.32	23.6	0.0048
5	5.6	0.39	28.8	0.0059
6	8.1	0.46	34.1	0.0070
7	11.5	0.53	39.6	0.0081
8	13.6	0.61	45.1	0.0093

4.3 Preliminary Experiments with Tissue Mimicking Phantom

Acoustically sensitive microcapsules were tested to see what effect, if any, an ultrasound pulse would have. The long term goal of these phantom experiments is to develop a microcapsule that can be manipulated by ultrasound to release drug payload at a

controlled rate. Microcapsules and tissue mimicking phantoms were tested under sonication by an ultrasound pulse from a 2.25 MHz transducer and the effects were recorded.

Two different sets of results are shown from two different dates. The same batch of microcapsules was used in the phantom sample on both days. Fig. 27 shows a microcapsule with surrounding gelatin. The Targesons do not appear to be visible in this image. This is important because any effects by acoustic radiation force cannot be seen. In Fig. 28, three different time periods are shown at the top left hand corner of this microcapsule. The membrane of the capsule appeared to bubble after about a minute of exposure to continuous wave 60 mV peak-to-peak input from the waveform generator. For a continuous wave, this produces 0.57 W electrical input power into the transducer from the arbitrary waveform generator and power amplifier. Acoustic power is 0.51 W assuming a 90% efficiency, I_{spta} is 6.65 W/cm^2 , and MI is 0.31.

In Fig. 29 the Targesons do not appear to be visible in this image. This is important because any effects by acoustic radiation force cannot be seen. At $t = 45 \text{ s}$, the membrane of the capsule appears to bulge suddenly. This bulging continued until $t = 60 \text{ s}$ when there appeared to be a break in the membrane.

Fig. 30 shows a microcapsule that was sonicated for one minute at 65 mV input peak-to-peak from the waveform generator. For a continuous wave, this produces 0.67 W input electrical power. Assuming 90% transducer efficiency, it produces an acoustic power of 0.60 W, a I_{spta} of 8.58 W/cm^2 and a mechanical index of 0.33. Four different time periods are shown of the top right corner of this microcapsule. The oval mark on the

right side of the capsule gradually shrunk over one minute of sonication. Without being able to view Targesons, it is difficult to determine what ultrasound mechanism may have caused these changes (see section 3.3.2).

Fig. 27. Microcapsule image taken from transmission light video microscope

More than likely, the prolonged exposure to high intensity continuous wave caused hyperthermia. These results are likely due to heat exposure because these effects were not seen until a prolonged period. After running this experiment, neither the phantom, the DI water, microcapsules, nor the plastic at the bottom of the stand-off were warm to the touch.

Fig. 28. Microcapsule membrane under sonication at different times

Fig. 29. Microcapsule image taken from transmission light video microscope

Fig. 30. Microcapsule membrane under sonication at different times

5. CONCLUSIONS AND FUTURE WORK

Multiple steps were performed to aid in the development of mass transport of drug for cancer therapy. The electrical input power and acoustic intensity measurements provided power and intensity information when the preliminary phantom measurement was performed.

The RF power measurement revealed that the combination of arbitrary waveform generator and RF power amplifier produced calibrated results corresponding to (7), the equation relating voltage and power. The Blackman window was ideal for calculating the power spectrum. Two different power equations, (20) and (21), were found based on varying the burst count and varying the input voltage. These equations can be used to calculate the electrical power input to the transducer for varying pulsing schemes in Table 6, efficiency calculations seen in Table 7, and safety parameter measurements seen in Table 8.

The acoustic pressure and intensity of the therapy transducer revealed contours and values that allowed safety parameters to be calculated. A powered second harmonic was seen at 70 μ and was expected due to irregularities in the transducer and by the ultrasound pulse propagating through water. Measurements of center frequency, pulse repetition frequency, and hydrophone sensitivity allowed calculations of the pulse intensity integral. From the pulse intensity integral, the following peak values were calculated at the focus for I_{spta} , I_{sppa} , and MI respectively: 0.0059 W/cm², 28.76 W/cm², 0.39 for a 50 mV peak-to-peak input from the waveform generator. Using Table 4 to compare accepted diagnostic levels, the calculated levels in this experiment are below the

values for I_{sppa} , I_{spta} and MI. So the system serves as a vehicle tool to measure key indices for different relevant pulsing schemes to ensure safety.

The optical measurements revealed that prolonged exposure to continuous wave ultrasound produces visible changes in the membrane of the microcapsule. The Targesons were not visible in the microscope images especially when combined with gelatin. In the first experiment, the membrane appeared to bubble and burst at $t=45$ s and $t=60$ s. In the second experiment, a mark on the microcapsule appeared to gradually shrink over a period of one minute. These effects are possibly due to heat exposure since they were only seen after a long ultrasound exposure.

There are many improvements that can be made in the future for these experiments. The electrical input power measurement should be expanded to test the efficiency of the transducer device. A radiation force balance can measure the actual output power emitted from the transducer. The peak input power measurement in this thesis will be compared to the radiation force balance measurements.

The acoustic measurements will be expanded to include many different pulsing schemes as the effect of the transducer beam is seen on the microcapsules. As the lab receives more funds, more therapy transducers and will be purchased and tested using the same hydrophone scanning method.

The optical experimental materials and setup needs to be improved in order to produce repeatable results. The phantom swells under exposure to water, which degrades the quality of the microscopic image. The image gets increasingly worse over the experimental session. Genipin can be put in the phantom material to prevent swelling.

Additionally, the phantom will include a coagulant so that it mimics the thermal properties of tissue when heated above 55° C.

The stand-off and support clamp stand are not sufficient to use in positioning the transducer over the phantom and microcapsule. In future experiments the phantom and microcapsule will be simultaneously sonicated and imaged. This will require a new apparatus to be designed and then built by the machinist at SJSU. The apparatus will include two threaded sockets for the imaging and therapy transducer that are angled at 45° to the microscope plate.

A biological microscope with a depth of field of 35 mm and a high speed camera is required to see clear effects of the ultrasound on the microcapsules. These experiments used a metallurgical microscope which only has a depth of field of 3 mm. This makes focusing on a microcapsule very difficult.

Work in the area of high intensity ultrasound for use in drug delivery methods will continue within the Electrical and General Engineering departments. As seen in Fig. 1, there are multiple stages just in the development of the transport method. If this experimental process yields positive results, further calibration and device development will be needed.

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