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Characterization of Cavitation Effects in Therapeutic Ultrasound: Sonophoresis Experiments and Quantitative Emission Measurements

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Characterization of cavitation effects in therapeutic ultrasound: sonophoresis experiments and quantitative emission measurements

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Abstract

Fundamental to the use of ultrasound for therapeutic benefit is a comprehensive understanding and identification of the underlying mechanisms. Specifically, consequential bioeffects during therapeutic ultrasound commonly coincide with the onset of microbubble cavitation, especially for drug-delivery applications. Hence, there is a need for monitoring and characterization techniques that provide quantitative metrics for assessing cavitation activity during ultrasound exposure in order to monitor treatment progress, identify interactions of cavitation with tissue, and provide dosimetry metrics for avoiding potentially harmful exposures both for therapeutic and diagnostic purposes.

The primary goal of the work presented in this dissertation was to characterize the role of cavitation during sonophoresis using quantitative and system-independent approaches. First, this goal was accomplished using traditional passive cavitation detection techniques to monitor cavitation emissions during in vitro intermediate-(IFS, insonation frequency $f_0 = 0.1–1 \text{ MHz}$) and high-frequency sonophoresis (HFS, $f_0 >1 \text{ MHz}$) treatments of in vitro porcine skin samples in Chapter 2. The relative intensity of subharmonic acoustic emissions from stable cavitation occurring near the skin surface was measured using a single-element PCD and was shown to correspond with reductions in skin resistivity, a surrogate measure of permeability, for all sonophoresis treatments. However, the acoustic emissions measured during
sonophoresis provided incommensurable quantities between the different treatment regimes due to unaccounted frequency-dependent variations in the sensitivity of the PCD and diffraction effects in the cavitation-radiated pressure field received by the PCD.

Second, methods were developed and employed to characterize the wideband absolute receive sensitivity of single-element focused and unfocused receivers in Chapter 3. By employing these characterization techniques and by accounting for the frequency-dependent response of the receiving system, the cavitation-radiated pressure incident on a PCD can be elicited from the system-measured voltage. Guidelines for accurate calibration measurements were established via simulation and the receive sensitivity of various PCDs were measured, including that of the PCD employed for sonophoresis in Chapter 2.

Third, in Chapter 4, a method for relating PCD-measured pressures, and by extension the system-measured voltage, to the acoustic power radiated by cavitation within a defined region of interest (ROI) was developed. This approach is accomplished by compensating PCD-measured pressures with a derived factor that accounts for the diffraction-dependent spatial variations in PCD sensitivity. The accuracy of this method was investigated via simulation. Further, this approach was employed to characterize the acoustic power radiated by stable cavitation over the skin surface during IFS and HFS using the system-dependent emission measurements made in Chapter 2, the PCD characterization conducted in Chapter 3, and the compensation factor calculated in Chapter 4.

The PCD calibration and measurement compensation methods developed here...
are broadly applicable for different single-element receivers, cavitation-monitoring applications, and frequencies. Hence, this approach enables a system-independent technique for characterization of cavitation-radiated acoustic powers, which may serve as a standard characterization technique.
Publications

The author of this dissertation has contributed to the following articles that are published in or submitted to peer-reviewed scientific journals:


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Chapter 1

Introduction

1.1 Background and significance

1.1.1 Overview: therapeutic ultrasound

Although commonly recognized for its uses in modern medicine as a diagnostic imaging modality, ultrasound was initially investigated and employed as a therapeutic tool. In the 1920’s Paul Langévin observed the destruction of schools of fish when passing through the beam of underwater sonar as well as noting pain induced in his hand when placed in an insonified water tank [Christian et al., 2014, Fyfe and Bullock, 1985]. These observations, made some 20 years prior to the initial implementations of ultrasound for diagnostic imaging [Dussik, 1942, Ludwig and Struthers, 1949], revealed the potent nature ultrasound could have on biological tissue. Additionally, these observations initiated research aimed at utilizing the associated biological effects for therapeutic treatment [Wood and Loomis, 1927].

Subsequent research lead to clinical investigations using ultrasound for various therapies [Lehmann, 1953] including neurosurgical [Fry et al., 1954] and drug delivery [Newman et al., 1958] applications as early as the 1950’s.

In recent years, the use of ultrasound for therapeutic purposes has been demonstrated for applications including the homogenization of tissue [Kieran et al., 2007], enhanced transport of drugs into various tissues [Mitragotri, 2005, Sutton et al., 2013], and increased rate of blood clot lysis [Datta et al., 2006, Bader et al., 2015]. Transcranially applied ultrasound for treatment of thrombotic diseases, such as stroke [Alexandrov et al., 2004], and the reduction of Parkinsonian tremor by lesioning of the thalamus [Elias et al., 2013] have been demonstrated in the clinic. Additionally, encouraging results in ultrasound-mediated drug delivery have led to FDA approval for clinical applications using ultrasound to enhance transdermal drug delivery [Maruani et al., 2012, Packer et al., 2013, Becker et al., 2005], as well as treatments for cancer and meningitis [Huang, 2008]. For other clinical applications, treatment of essential tremor, bone metastasis, ablation of prostate and uterine fibroids using thermal ablation have been recently approved by the U.S. Food and Drug Administration (FDA) [Miller et al., 2012]. Outside the U.S., the treatment of breast, kidney, and liver cancers by ultrasound have also been employed clinically [Miller et al., 2012].

The prospective of new therapeutic ultrasound applications is especially auspicious. However, fundamental to these treatments is a comprehensive understanding and identification of the underlying mechanisms in order to better induce and exploit desired bioeffects, meanwhile avoiding undesirable effects and
maintaining its noninvasive status. In addition to understanding the associated mechanisms, proper monitoring and measuring techniques are required to assess treatment progress and provide dosimetry metrics for avoiding potentially harmful exposures both for treatment and for diagnostic purposes.

1.1.2 Adverse bioeffects induced by ultrasound

Investigations of adverse and therapeutic biophysical effects induced by ultrasound have been ongoing since the 1920s [Fyfe and Bullock, 1985]. Adverse bioeffects due to ultrasound exposure primarily arise from either thermal or mechanical mechanisms. Specifically, thermal effects can occur as the mechanical energy of the ultrasound wave propagating through tissue or fluid is absorbed and converted to thermal energy, resulting in a subsequent local temperature elevation. Although moderate temperature increases ($\Delta T < 1.5 \, ^\circ\text{C}$) likely have no adverse effect on living tissue even for prolonged exposures [Barnett et al., 2000], moderate temperature increases ($\Delta T > 4.5 \, ^\circ\text{C}$) can cause irreversible damage, for example to developing embryos [Edwards et al., 1995]. Additionally, hyperthermic temperature increases induced by ultrasound can lead to thermal fixation ($43 < T < 100 \, ^\circ\text{C}$) and coagulative necrosis ($T > 100^\circ \text{C}$) of tissue [Wu et al., 2006, Yu et al., 2015].

Adverse bioeffects due to ultrasound-induced mechanical tissue damage are most commonly associated with the spontaneous nucleation and acoustic excitation of gaseous bubbles, a phenomenon referred to as acoustic cavitation. For acoustically driven bubbles, referred to hereafter as cavitating bubbles, the dynamical response to ultrasound exposure includes bubble growth, sustained radial oscillations, and collapse. Physical effects associated with the dynamical response of cavitating
bubbles include fluid jetting and microstreaming, thereby inducing mechanical stress on surrounding tissues, or even result in the production of free radicals [O’Brien, 2007]. In particular, the unintended occurrence of cavitation within tissue or blood can lead to cellular lysis [Vivino et al., 1985], as well the onset of hemorrhage in the lungs [Holland et al., 1996, Dalecki et al., 1997], kidneys [Wible et al., 2002], and blood vessels [Miller and Gies, 1998, Chen et al., 2010] due to the physical effects associated with cavitating bubbles. However, cavitation activity is typically initiated randomly and spontaneously [Church, 2002] and its occurrence is exceedingly rare and highly localized [Carstensen, 1987] due to the sparse population of stabilized nuclei in biological tissues [Fry et al., 1995]. Because of these factors, the onset of cavitation in biological tissue is difficult to obtain unless the acoustic intensity of the applied ultrasound is sufficiently high.

In addition, significant advances in recent years have been made to avoid these adverse bioeffects in order to ensure the safety and non-invasive nature of ultrasound for diagnostic applications in vivo. In particular, metrics for assessing the potential for adverse thermal [NEMA, 2004, Bigelow et al., 2011] and mechanical bioeffects [Apfel and Holland, 1991] have been developed and employed on clinical scanners. The potential for thermal effects due to tissue heating from absorption of acoustic energy increases with frequency, intensity and duration of ultrasound exposure. Similarly, the onset of cavitation activity that may lead to mechanical bioeffects is characterized by a threshold [Holland and Apfel, 1989, Yang and Church, 2005] that increases proportionally with the pulse length and peak negative pressure, and inversely with the square root of the frequency of applied ultrasound [Fowlkes
and Crum, 1988, Apfel and Holland, 1991, Bader and Holland, 2012]. Hence, ultrasound in the megahertz frequency range employed for diagnostic purposes is typically applied at relatively low intensities (0.5-50 mW/cm$^2$) and in short pulses (microseconds) in order to minimize the potential for adverse bioeffects associated with mechanical and thermal mechanisms. Although these metrics provide reliable guidelines for avoidance of adverse bioeffects, these do not serve as metrics for quantifying or monitoring of adverse effects should they occur.

### 1.1.3 Therapeutic bioeffects induced by ultrasound

Although linked to unwanted bioeffects during diagnostic ultrasound, thermal and mechanical mechanisms may be elicited during ultrasound treatments, under controlled conditions, to exploit the associated bioeffects for therapeutic advantage. Within the diagnostic range of acoustic intensities of less than 50 mW/cm$^2$, the interaction of acoustic ultrasound waves and biological tissue is relatively inert. However, mild therapeutic effects have been obtained in this range for applications such as improving the analgesic effect of local lidocaine delivery due to convective streaming ($0.17$ mW/cm$^2$, $f_0 = 48$ kHz) [Tachibana and Tachibana, 1993] and accelerated healing of bone fractures by stimulating mechanoreceptors at the cellular level ($30$ mW/cm$^2$, $f_0 = 1.5$ MHz) [Harrison et al., 2016]. These applications, due to their low applied acoustic intensities, typically present minimal opportunity for tangential or negative bioeffects.

In comparison, many therapeutic ultrasound treatments employ higher time-average acoustic intensities ranging from 0.5–100+ W/cm$^2$. For these applications, the thermal and mechanical bioeffects avoided in diagnostic applications are
deliberately elicited in a controlled manner to facilitate the given treatment. For example, high intensity focused ultrasound (HIFU) is employed at intensities typically greater than 1000+ W/cm$^2$. For these applications, HIFU is employed as a noninvasive and extracorporeal method to induce rapid tissue heating, capable of occluding blood vessels [Chen et al., 2003b, Hoerig et al., 2014] and ablating tissue at depth in highly localized regions (on the order of 10 mm$^3$) as an alternative to surgical resections of diseased tissue [Bailey et al., 2003, Mast et al., 2008b]. Thermal destruction of tissue due to HIFU exposure is accomplished by depositing large amounts of acoustic energy into a well-defined volume that induces severe temperature increases due to thermoviscous absorption of the mechanical ultrasound wave by tissue. The resulting temperature increases can result in thermal necrosis of cells in viable and malignant tissues within the target volume, while avoiding peripheral damage [Kennedy, 2005, Wu et al., 2006, Yu et al., 2015].

Similar to HIFU, another alternative to surgical resection of diseased tissues is a treatment termed histotripsy that is capable of destroying tissue within an isolated and targeted volume while sparing peripheral tissue [Xu et al., 2004, Roberts et al., 2006]. Histotripsy is conducted by applying extracorporeal ultrasound with higher acoustic intensities than even HIFU, typically greater than 20 kW/cm$^2$, and with comparatively shorter duration exposures (microseconds versus seconds). The mechanism of action for histotripsy is primarily unrelated to tissue heating, unlike that of HIFU. Rather, local tissue removal is achieved via non-thermal fragmentation at the subcellular level as a result tissue interacting with the mechanical action of acoustic cavitation [Parsons et al., 2006, Xu et al., 2007, Bader and Holland, 2016].
Acoustic cavitation in a broad sense refers to gas-filled microbubble activity within tissue or fluid that is initiated and driven by ultrasound exposure. Although cavitation is capable of providing thermal effects by enhancing tissue heating [Sokka et al., 2003, Farny et al., 2010], the mechanical properties associated with cavitation are often of most interest for therapeutic applications, especially for drug delivery. For example, the mechanical action of cavitating bubbles has been shown to lead to increased drug penetration rates for many ultrasound-enhanced drug delivery applications by disrupting various biological barriers [Ghosh et al., 1997, Husseini et al., 2005, Mitragotri, 2005, Sutton et al., 2013] as well as assisting in the targeted delivery or release of therapeutic molecules [Unger et al., 2004], genes [Chen et al., 2003a, Price and Kaul, 2002], and chemotherapeutics [Rapoport et al., 2009]. The various bioeffects associated with ultrasound- and cavitation-mediated drug delivery are brought on by non-thermal mechanisms including radiation force, microstreaming, shock waves, free radicals, microjets, and strain that are associated with different types of cavitation activity [O’Brien, 2007].

1.1.4 Acoustic cavitation: types and associated mechanisms

Acoustic cavitation generally refers to the linear or non-linear volumetric expansion and contraction of gas-filled, nano- to micron-sized bubbles within a fluid or tissue exposed to ultrasound [Leighton, 1994]. As an acoustic wave passes through a medium, nucleated bubbles of any dimension will expand within the rarefractional and contract within the compressional portions of the applied alternating pressure wave. The dynamic response of a cavitating bubble to the applied pressure wave is dictated by, among other factors, its size and stability, as well as the insonation
frequency and pressure [Church, 2002, Yang and Church, 2005]. At relatively low applied pressure amplitudes, a cavitating bubble may stably and repetitively pulsate volumetrically. This particular dynamic response of a cavitating bubble is termed ‘stable cavitation’. The radial pulsations of stable cavitation result in the generation of subharmonic acoustic emissions [Eller and Flynn, 1968, Leighton, 1994] and high-velocity microstreams around the oscillatory boundary layer of the cavitating bubble [Elder, 1959]. The onset of radial pulsations indicative of stable cavitation can occur at any applied pressure level, however the non-linear amplitude of the bubble oscillation and subsequent generation of subharmonic acoustic emissions generally increases with applied pressure amplitude and decreasing insonation frequency [Bader and Holland, 2012].

The amplitude of bubble oscillations increases with the intensity of the applied ultrasound until the growth of a bubble approaches an unstable threshold. Beyond this threshold, the inward moving fluid at the bubble wall has sufficient inertia to overcome any further expansion, and the bubble rapidly collapses [Leighton, 1994]. This cavitation activity is referred to as ‘inertial cavitation’ [Apfel, 1997]. Inertial cavitation can lead to fluidic microjet [Prosperetti, 1984] and shock wave [Pecha and Gompf, 2000] formations as well as the generation of broadband acoustic noise due to the supersonic acceleration of the bubble wall during collapse [Leighton, 1994].

1.1.5 Enhanced drug delivery mediated by ultrasound-induced cavitation

In comparison to stable cavitation, the energy, mechanisms, and corresponding bioeffects associated with inertial cavitation are typically more severe. For example,
inertial cavitation has been shown to produce free radicals [Flynn, 1964], erode solid materials [Tomita and Shima, 1986], and generate fluidic microjet and shockwave formations [Prosperetti, 1984, Pecha and Gompf, 2000, Brennen, 2013]. In tissue, these mechanisms may lead to detrimental bioeffects including blood vessel invagination [Chen et al., 2010], lysis of cells [Ward et al., 2000], lung damage and petechiae [Chaussy et al., 1982]. Yet, under controlled conditions, these mechanisms have the potential to provide beneficial bioeffects for drug delivery applications by permeabilizing and enhancing drug penetration across biological membranes associated with individual cells (sonoporation) [Taniyama et al., 2002, Zhou et al., 2008] and skin (low-frequency sonophoresis; LFS) [Tezel et al., 2002, Polat et al., 2011, Tezel and Mitragotri, 2003]. However, challenges exist in precisely controlling inertial cavitation, as its occurrence is typically transient in nature especially in vivo, making it difficult to provide desired bioeffects sustainably while avoiding detrimental bioeffects in adjacent tissue or cells.

Stable cavitation, on the other hand, can be initiated at significantly lower acoustic pressures and the associated bioeffects are generally considered less severe than those of inertial cavitation, yet the role of stable cavitation in ultrasound-mediated drug delivery is significant. For example, cavitating bubbles undergoing relatively low-amplitude, sustained volumetric oscillations may increase the rate of transport of oxygen and nutrients to some cells [Pitt et al., 2004, Pitt and Ross, 2003]. Cavitating bubbles undergoing relatively large and sustained radial pulsations can lead to drug release from various vesicles, providing localized delivery of therapeutics [Pitt et al., 2004, Husseini et al., 2005]. Stable cavitation is also
believed to play a significant role in the disruption of biological membranes, which is often temporary and reversible, facilitating ultrasound-enhanced drug delivery across the blood brain barrier [O’Reilly and Hynynen, 2012] and cellular membranes [Guzman et al., 2001, Wu and Nyborg, 2008]. Mechanistically, permeabilization of these biological membranes is generally believed to be achieved via high velocity gradients and hydrodynamic shear stresses induced on adjacent tissue boundaries by microstreaming around the oscillatory boundary layer of a neighboring bubble that is undergoing stable cavitation due to acoustic excitation [Williams, 1973]. In a similar manner, microstreaming from a bubble that is undergoing stable cavitation due to acoustic excitation can facilitate penetration of tissue plasminogen activator into and remove fibrin degradation products away from thrombi resulting in increased clot dissolution during ultrasound-enhanced thrombolysis [Datta et al., 2006, Sutton et al., 2013]. Considering the compelling role identified for stable cavitation in these drug delivery applications, it is likely that stable cavitation also plays a significant role in permeabilizing other biological barriers for applications yet to be identified or explored, such as enhanced delivery of topically applied drugs to the skin.

1.1.6 The skin barrier to drug delivery

Dermal and transdermal drug delivery refers to the topical administration strategy employed for transporting medications across the skin barrier for local delivery to treat viable skin layers or delivery to underlying vasculature for systemic absorption. This approach provides an alternative strategy to traditional delivery routes with several distinct advantages in that the skin presents a large (1–2 m²) and easily accessible surface area, avoids premature degradation and first-pass gastrointestinal
or hepatic metabolism, and offers the potential for rate-controlled delivery [Naik et al., 2000, Mitragotri, 2004, Prausnitz et al., 2004]. Especially appealing is the potential to replace hypodermic needles, notably for applications that require repeated delivery such as insulin for diabetic patients or vaccination delivery in children [Tachibana and Tachibana, 1991, Martanto et al., 2004]. In addition to improving patient compliance for these applications, this alternative route may reduce the risk of disease transmission by reuse of needles, especially in developing countries [Miller and Pisani, 1999], and provide some vaccinations the added benefit of eliciting a greater immune response by targeting the Langerhans cells in the skin [Foldvari et al., 2006].

However, successful delivery across the skin is typically achieved unaided only for drugs that have molecular masses less than 500 Daltons, are highly lipophilic, and require doses of milligrams per day or less such as scopolamine, nicotine, lidocaine, and estrogen. [Guy et al., 1987, Bos and Meinardi, 2000, Prausnitz et al., 2004]. This limitation to drug penetration is almost exclusively a result of the barrier properties presented by the skin itself. Specifically and quite impressively, the primary resistor to drug penetration is the thinnest (10-20 micron) and outermost skin layer, the stratum corneum [Elias, 1983]. The architecture of this layer, often referred to analogously as a ‘brick and mortar’ structure, provides the skin its barrier properties [Potts and Francoeur, 1991]. This structure is composed of a laminate of compressed brick-like keratin-filled corneocytes embedded in a mortar-like continuum of multilamellar sheets of lipids [Christophers, 1971, Elias, 1981], resulting in a highly tortuous lipoidal diffusion pathway that drugs are required
to penetrate before even reaching the outermost viable skin layers. Hence, recent investigations have been aimed at modifying the stratum corneum architecture in order to increase the number and types of drugs which have otherwise presented poor permeation profiles through the skin [Prausnitz and Langer, 2008].

Various mechanical and chemical approaches have been investigated for the purpose of increasing skin permeability to drugs by modifying the stratum corneum architecture, often transiently and reversibly. The use of chemical penetration enhancers has been shown to be effective for enhancing the permeability of skin to various molecules, however this approach has had little impact on the delivery of higher molecular weight permeants [Ghosh et al., 1997, Prausnitz et al., 2004]. One mechanical approach is the application of a continuous low-voltage current across the skin (iontophoresis). However, this approach is only effective for charged molecules and is limited to penetration across the stratum corneum only when the electric field is applied, requiring continuous application in order to obtain sustained delivery [Kalia et al., 2004, Prausnitz and Langer, 2008]. Alternatively, the application of ultrasound (sonophoresis) to skin has been shown to be effective at increasing the transdermal penetration rate of various permeants including high-molecular weight drugs, hormones, fibers, biopolymers, oligonucleotides, liposomes, nanoparticles, and even vaccines [Polat et al., 2010, Polat et al., 2011]. Additionally, sonophoresis is capable of providing prolonged and sustained delivery, even after treatment, while also potentially providing a safe treatment since the modifications made to the skin barrier may be reversible [Mitragotri, 2004, Polat et al., 2011].
1.1.7 Sonophoresis: ultrasound-enhanced transdermal drug delivery

Due to the robust barrier presented by the outermost skin layer, the stratum corneum (SC), a variety of non-invasive technologies have been investigated with the aim of increasing skin permeability by transiently perturbing the SC architecture. Among these technologies, the application of therapeutic ultrasound to skin, a treatment termed sonophoresis, has proven to be particularly promising.

The primary enhancement mechanism of sonophoresis is believed to be acoustic cavitation. Increased skin permeability can be achieved when cavitating bubbles interact with and modify the skin barrier by inducing, dilating, and connecting defects [Wu et al., 1998, Paliwal et al., 2006, Mitragotri et al., 1995b] to form regions of increased permeability within the SC [Tang et al., 2002a, Tezel et al., 2001]. However, the fundamental mechanisms have yet to be fully understood and characterized. These mechanisms include the specific location(s) and type(s) of cavitation responsible for skin permeabilization. Additionally, these mechanisms may depend on the frequency of ultrasound used for sonophoresis since higher ultrasound frequencies result in cavitating bubbles with smaller dimensions and different dynamics than those produced by lower frequencies [Yang et al., 2004].

In a study designed to identify the location of permeability-enhancing cavitation during low-frequency sonophoresis (LFS, <100 kHz), skin electrical resistance, used as a surrogate measure of skin permeability, was shown to be significantly reduced only when cavitation was present within the donor medium outside of skin [Tang et al., 2002b]. Moreover, this study demonstrated
that cavitation within the skin was unlikely to occur or provide a significant
effect on skin permeability during LFS. Investigations aimed at identifying the
critical types of permeability-enhancing cavitation during LFS have demonstrated
a strong correlation between enhancement and measured broadband acoustic
emissions, indicating that inertial cavitation occurring outside of the skin is the
primary permeabilization mechanism of LFS [Ueda et al., 2009, Tezel et al.,
2002, Tang et al., 2002b]. Furthermore, theoretical [Tezel and Mitragotri, 2003]
and experimental [Wolloch and Kost, 2010] investigations have indicated that
the permeabilization effect from inertial cavitation during LFS is primarily due
to microjet formations, manifested during the asymmetric collapse of cavitating
bubbles near the skin surface, that impact and locally perturb the SC. Exploitation
of this well characterized mechanism has led LFS treatments to provide a greater,
although reversible, permeabilization effect while also significantly reducing
treatment times [Terahara et al., 2002, Schoellhammer et al., 2012].

The role of cavitation during intermediate- (IFS, 100–1000 kHz) and high-
frequency sonophoresis (HFS, > 1 MHz) is likely different than during LFS.
Among studies investigating IFS, Wu et al. identified the existence of randomly-
arranged, air-filled voids approximately 20 µm in diameter within the SC of human
skin, apparently due to cavitation, after in vitro exposure to 168-kHz ultrasound
[Wu et al., 1998]. In another study, increased enhancement of skin permeability was
shown by Ueda et al. to scale directly with rising broadband emissions, emanating
from inertial cavitation presumed to be occurring within the donor medium outside
the skin, during sonophoresis using frequencies as high as 445 kHz [Ueda et al.,
Cavitation within the skin may play a greater role during HFS because at higher frequencies the resonant diameters of cavitating bubbles are smaller, comparable to dimensions of the lacunar voids within the skin where cavitation can occur [Simonin, 1995, Bommannan et al., 1992, Menon et al., 1994]. This assertion was investigated experimentally in a study by Mitragotri et al., showing that significant changes in skin electrical resistance occurred when the potential for cavitation was isolated to within the skin [Mitragotri et al., 1995b]. Microscopy-based analysis of skin after HFS in this study identified a disarrangement of the SC lipid bilayer, further indicating that skin permeabilization was due to cavitation occurring within voids near the corneocytes of the SC. In a study by Park et al., penetration of fluorescein isothiocyanate (FITC)-dextrans across skin during HFS was significantly enhanced when cavitation nuclei, in the form of ultrasound contrast agents (UCA), were introduced to the surrounding medium [Park et al., 2012]. Due to the relatively low pressures used for HFS in this study, the observed enhancement was suggested by these authors to be caused by microstreaming associated with stable cavitation occurring outside the skin.

1.1.8 Cavitation monitoring techniques

Metrics employed for assessing the potential for thermal [NEMA, 2004, Bigelow et al., 2011] and mechanical bioeffects [Apfel and Holland, 1991] for clinical imaging applications only provide an estimate of the onset of adverse bioeffects based on the insonation parameters, and do not provide characterization or monitoring of the mechanisms or associated bioeffects induced during therapeutic applications.
Therefore, various techniques have been developed for monitoring thermal effects and assessing treatment progress during *in vivo* thermal ablation using ultrasound [Mast et al., 2008a, Subramanian et al., 2014] and magnetic resonance (MR) imaging [Hynynen et al., 1996, Hynynen and McDannold, 2004]. Similar to thermal therapies, monitoring is essential for assessing the safety, progress, and efficacy of treatments, as well as to provide mechanistic insight into the interaction of cavitation with tissue during cavitation-based therapies. However, the approaches used to monitor thermal effects are not capable of monitoring cavitation-based ultrasound therapies because of the inability to image cavitation activity in real-time with MRI [Damianou et al., 2004] and the potential for the treatment beam and emissions from cavitation to interfere with B-mode ultrasound imaging [Gyöngy and Coussios, 2010b]. Hence, various alternative monitoring technologies have been developed in order to obtain remote feedback of cavitation activity.

One approach to monitoring cavitation activity employs the use of high-speed cameras to facilitate direct optical measurements. This approach has provided fundamental insights into the dynamic response of single microbubbles to ultrasound [Chomas et al., 2001, de Jong et al., 2000], as well as enabling visualization of bubble clouds generated during therapies such as histotripsy [Xu et al., 2007, Maxwell et al., 2013]. In addition, optical approaches for therapies conducted *in vitro* have provided fundamental mechanistic insights about the interaction of cavitation with biological tissues such as microvessels [Caskey et al., 2007, Chen et al., 2011], individual cells [Ohl et al., 2006] and blood clots [Bader et al., 2015] during sonoporation and sonothrombolysis, respectively. Although optical methods provide
direct observations of cavitation, this approach is only capable of monitoring cavitation over a short period of time at depths less than a few centimeters into tissue [Maxwell et al., 2013], making it a nonviable method for monitoring cavitation during treatments conducted *in vivo* or for cavitation activity occurring within opaque tissues.

As a cavitating bubble oscillates or collapses, it perturbs the surrounding medium and radiates its own characteristic acoustic pressure field. The radiated pressure field can be monitored using a secondary transducer in receive-only mode as a passive cavitation detector (PCD) to effectively ‘listen’ to the sound generated by cavitation in real time [Roy et al., 1990]. This acoustic measurement technique is advantageous as a non-invasive monitoring approach due to its ability to interrogate cavitation activity at depth within tissue and other opaque media without perturbing the treatment beam, medium under treatment, or the cavitating bubbles. Passive techniques have therefore been employed in various studies investigating the dynamics of cavitation [Collin and Coussios, 2011, Radhakrishnan et al., 2013, Vlaisavljevich et al., 2015], interaction of cavitation with various tissues and cells [Hallow et al., 2006, Chen et al., 2003c, Hwang et al., 2006], and used to estimate cavitation thresholds [Holland and Apfel, 1990, Khokhlova et al., 2009, Li et al., 2014, Gruber et al., 2014].

Passive monitoring of cavitation emissions is conducted using the PCD to sense the cavitation-radiated pressure incident on its active element. The energy of the measured instantaneous pressure wave is mitigated by the frequency-dependent receive sensitivity of the PCD and converted to an electrical signal that is digitized...
and stored by the receiving system. Spectral analysis of the acquired voltage signal is typically conducted to characterize the relative presence, type, and intensity of cavitation activity by identifying characteristic frequency content associated with each cavitation type. Specifically, emissions within the subharmonic (half the insonation frequency) band of the received signal are indicative of stable cavitation, resulting from the repetitive nonlinear oscillations of the bubble [Eller and Flynn, 1968, Leighton, 1994]. Inertial cavitation is identified by an increase in overall received signal level as broadband noise, due to the impulse-like pressure radiated upon the high velocity collapse of the bubble wall [Leighton, 1994].

For passive detection of emissions from either type of cavitation activity, measurements are commonly quantified by temporally integrating the amplitude [Chen et al., 2003c], squared amplitude [Hoerig et al., 2014], or decibel-scaled level [Hallow et al., 2006, Mast et al., 2008b] of the system-measured voltage signal within distinct frequency bands associated with cavitation. Although these are system-dependent measurements, this approach has been used to provide critical information for establishing relationships between cavitation and \textit{in vitro} bioeffects. For example, PCD-measured subharmonic emissions from stable cavitation have been associated with enhanced thrombolysis [Datta et al., 2006] or cellular damage [Morton et al., 1983], as well as broadband emissions from inertial cavitation with enhanced skin [Tezel et al., 2002] and cellular [Hallow et al., 2006] permeabilization.

Transducers employed as a PCD are commonly composed of a single element, which is either flat and unfocused or curved and geometrically focused. Both types of transducers provide a fixed spatial sensitivity pattern that is dependent on the
frequency and geometry of the transducer’s active element [Rayleigh, 1945]. Focused transducers are often employed due to their comparatively greater spatial specificity and significantly greater sensitivity, albeit over a substantially smaller volume than that provided by an unfocused transducer. The broader beamwidth of unfocused transducers allows a greater volume of interest to be interrogated, but this comes at the cost of decreased spatial resolution and comparatively lower sensitivity than focused transducers.

One primary limitation associated with employing single-element transducers for cavitation monitoring is the inability to spatially resolve emissions generated by individual bubbles undergoing cavitation except when the bubble position is isolated to within the focal region of the transducer. Hence, the ability to differentiate signals received by spatially distributed sources such as cavitating bubbles is limited and spatial registration of cavitation activity and associated bioeffects is not possible. This limitation can potentially be circumvented by the use of an array of elements to map cavitating and emission generating bubbles passively, yielding temporal and spatial information of cavitation [Farny et al., 2009, Salgaonkar et al., 2009, Gateau et al., 2011]. However, in addition to the higher cost in comparison to single-element configurations, array-based methods require the use of more complex hardware and software. Moreover, precise spatial registration of cavitation is generally limited to the transverse direction of the array as axial resolution is diffraction limited due to the fixed, finite size of the array subaperatures, resulting in image degradation. To address these issues, recent studies have investigated improved imaging algorithms to improve axial resolution [Haworth et al., 2014, Coviello et al., 2015].
1.1.9 Cavitation characterization and quantitative emission measurements

In addition to the limitations previously described for passive monitoring of cavitation emissions, the voltage signals used for analysis are acquired as system-dependent measurements. Acquisition is achieved first by the phase-sensitive element receiving the complex pressure incident on and averaged over its surface [Chen et al., 1997]. Hence, the received pressure may be influenced by phase cancellation over its surface depending on its size and shape, its position relative to cavitating bubbles, and the frequency of emissions. Second, the received pressure is converted to a voltage by the receiving element and acquired by the electronics of the receiving system. The PCD and receiving system individually influence the measured voltage by their respective frequency-dependent sensitivities, including any gain or filtering applied to the acquired voltage signal. Finally, the system-acquired voltage signal is typically analyzed to represent the acoustic power radiated by cavitation. This analysis is typically conducted without compensation for the various frequency-dependent modifications made to the signal or diffraction effects in the cavitation-radiated field, making it challenging to compare measurements made using different receivers, configurations, or analysis techniques. Therefore, traditional passive cavitation emission monitoring techniques result in relative measurements that are provided in terms of different fundamental quantities than those of the cavitating bubbles and do not directly represent the local power or energy delivered to tissue by cavitation.

For single-element receivers, absolute measurements of the pressure incident
on its surface from cavitation may provide useful information about the observed cavitation activity. However, this approach requires characterization of the PCD and other components of the receiving system. Despite a number of available methods for characterizing the receive sensitivity of small needle-type receivers [Ludwig and Brendel, 1988, Smith and Bacon, 1990, Labuda et al., 2004], there is a significant absence of well established techniques for characterizing larger and non-flat aperture transducers commonly employed as a PCD. Additionally, absolute measurements are only viable when cavitation activity can be isolated at the focal region of the element in order for the received pressure to arrive uniformly over its surface. Otherwise, the receiver-measured pressure will be less than the expected value due to phase cancellation from diffraction in the cavitation-radiated field. For example, absolute measurements provide a viable characterization approach for single-bubble cavitation experiments, when the position of a cavitating bubble can be precisely known and maintained such that diffraction effects are negligible [Collin and Coussios, 2011]. However, cavitation activity typically occurs with spatiotemporal stochasticity and the distribution of cavitating bubbles at any point in time is not readily predictable [Maxwell et al., 2013]. Hence, even for absolute emission measurements, challenges still exist in accounting for diffraction effects using single-element receivers.

Alternatively, array-based mapping of acoustic emissions has been demonstrated to be capable of distinguishing emissions generated by individual bubbles undergoing cavitation, allowing the acoustic power or energy radiated by individual bubbles to be estimated [Gyöngy and Coussios, 2010b, Gyöngy and Coussios, 2010a, Choi
et al., 2014]. However, the results of this approach become dubious when multiple cavitating bubbles are positioned adjacent temporally and spatially such that the corresponding point-spread-functions overlap and energy is mapped incorrectly to locations where there are no bubbles [Haworth et al., 2017]. Hence, identifying the precise locations of cavitation in order to determine its radiated power remains an ongoing challenge that is not immediately mitigated by array-based detection, despite its greatly improved spatial specificity in comparison to single-element detectors.

1.2 Gaps in knowledge

Acoustic cavitation plays a critical role during many ultrasound-enhanced therapeutic and drug delivery applications. Sonophoresis is one such application that employs ultrasound to enhance percutaneous drug absorption. For sonophoresis, the interaction of cavitation with skin is believed to lead to increased permeability by altering the structure of the stratum corneum. However, the specific cavitation mechanisms associated with permeabilization have not been fully elucidated and are consequently unmanageable for applications employing intermediate- (IFS, insonation frequency $f_0 = 0.1–1$ MHz) and high-frequency (HFS, $f_0 >1$ MHz) ultrasound for sonophoresis.

Identification of the specific cavitation types associated with IFS- and HFS-enhanced skin permeability may be conducted by analyzing cavitation emission measurements made using a single-element transducer in receive-only mode as a passive cavitation detector (PCD). However, under traditional measurement and
analysis techniques, emission measurements are frequency- and system-dependent. Hence, emissions measured during IFS are not comparable with those made during HFS treatments. This complication persists in other cavitation-based studies as well, as any modification to the test configuration, PCD, system-electronics, or analysis method produces unique results. Additionally, from a dosimetry perspective for therapeutic applications, the arbitrary dimensions of the analyzed PCD-measured signals provide no direct information about the acoustic energies the treated tissues are exposed to.

One approach to overcoming these challenges is to characterize cavitation directly by its acoustic power radiated within a given treatment region of interest (ROI). First, this approach requires compensation of the measured signals for the sensitivities of the PCD and receiving electronics in order to determine the average incident pressure from cavitation over the PCD element. However, although characterizing the frequency response of electrical components of the receive system is straightforward, broadly applicable techniques for characterizing the receive sensitivity of transducers commonly employed as PCDs are not widely available and are especially challenging for focused receivers.

Second, due to phase effects in the cavitation-radiated field, PCD-received pressures are influenced and require further compensation to account for diffraction. However, accounting for diffraction can only be readily and directly conducted if the PCD-generated signals from specific cavitation events can be spatially and temporally distinguished, which is challenging due to the stochastic nature of cavitation and fixed spatial sensitivity of single-element receivers. The development
of compensation factors accounting for on-average diffraction effects in the cavitation-radiated pressure field have yet to be determined for single-element PCDs.

Direct characterization of cavitation-radiated powers from PCD-measured signals would facilitate the potential for standardized measurement and analysis techniques. From this approach, for example, a greater mechanistic understanding of the interaction of cavitation with skin could be gained as the cavitation-radiated power required to induce changes in skin resistance during sonophoresis or other bioeffects could be determined. Because the role of cavitation during sonophoresis is potentially frequency-dependent, the role of cavitation may also be distinguished between different treatment regimes. In addition to providing mechanistic insights to guide future sonophoresis treatments, this approach could potentially serve as a standard metric for quantifying cavitation activity for other cavitation-based therapies. Specifically, the occurrence of various bioeffects induced during ultrasound treatments is likely related to the energy delivered by cavitation to tissue. This approach could be used as a metric for assessing the safety of treatments as well as a standard for estimating the progress of a given treatment.

1.3 Hypothesis and Specific Aims

The primary goal of the studies described in this dissertation was to address the gaps in knowledge regarding: (1) the mechanistic role of acoustic cavitation during sonophoresis, specifically for IFS and HFS applications, and (2) system-independent methods for quantitative characterization of cavitation activity. The research plan summarized in the following hypotheses and specific aims was developed to obtain
this goal:

**Hypothesis 1**: Ultrasound-induced acoustic cavitation is the primary mechanism of permeability-enhancing perturbations made to skin during sonophoresis.

**Specific Aim 1**: Identify the specific location(s) and type(s) of acoustic cavitation that lead to increases in skin permeability during intermediate- (IFS) and high-frequency sonophoresis (HFS).

**Hypothesis 2**: Changes in skin permeability during sonophoresis correlate with the acoustic power radiated by cavitation on the skin surface.

**Specific Aim 2(a)**: Establish and validate methods to calibrate the absolute receive sensitivity of single-element passive receivers in order to relate transducer-generated voltages to received pressures.

**Specific Aim 2(b)**: Employ calibration methods to characterize the absolute receive sensitivity of the PCD system used for sonophoresis and determine the PCD-received acoustic pressures from cavitation.

**Specific Aim 3(a)**: Establish methods for correcting PCD measured pressures to account for diffraction effects in order to estimate the acoustic power radiated by cavitation from a defined region of interest.

**Specific Aim 3(b)**: Correlate changes in skin resistance to the acoustic power radiated from cavitation on the skin surface during IFS and HFS.
1.4 Dissertation overview

The body of this dissertation describes experiments, methods, simulations, and theory relating to cavitation detection, quantification, and the identification of mechanisms associated with therapeutic ultrasound applications such as sonophoresis. Specifically, Chapter 2 describes a study that was employed to elucidate the specific cavitation mechanisms, namely the precise locations and types of cavitation, associated with increased skin permeability resulting from intermediate- (IFS) and high-frequency sonophoresis (HFS) (Specific Aim 1).

In this chapter, cavitation was isolated to specific locations relative to in vitro porcine skin samples during sonophoresis treatments. A single-element PCD was used to monitor cavitation emissions and spectral analysis was conducted on the measurements in order to delineate the types and relative intensity of cavitation that occurred during sonophoresis. These results were compared to concurrently measured changes in skin resistance, which served as a surrogate and instantaneous measure of skin permeability, in order to identify the types and locations of permeability-enhancing cavitation. The results of this study may be used to guide treatment design, including ultrasound exposure parameters, employed for future treatments.

In Chapter 3 and Chapter 4 techniques for quantitatively characterizing the acoustic properties of cavitation from passively measured acoustic emissions were developed. In Chapter 3, techniques for characterizing the frequency-dependent receive sensitivity of single-element transducers were evaluated, developed, and the accuracy of each analyzed using numerical simulations and experimental
measurements. These techniques allow the conversion of the measured voltage to the absolute pressure incident on a PCD element from acoustic sources such as cavitating bubbles (Specific Aim 2). The techniques developed in this chapter were validated using numerical simulations and experimental measurements. In addition, these techniques were employed to characterize the receive sensitivity of a focused and an unfocused single-element receiver. Specifically, the unfocused PCD that was calibrated was that used to make emission measurements in Chapter 2, enabling calculation of the cavitation-radiated pressures received by the PCD during sonophoresis.

Chapter 4 describes theory, simulations, and measurement techniques employed for characterizing the acoustic power radiated by cavitation within a defined region of interest (ROI). This characterization approach builds upon Chapter 3 by relating acoustic pressures measured by a calibrated PCD to the average cavitation-generated acoustic power by compensating PCD-measured pressures with a derived diffraction correction factor. The diffraction correction factor was derived by estimating the average spatial sensitivity of a PCD over a defined ROI in order to compensate for on-average diffraction effects in the cavitation-radiated pressure field over the surface of a single-element PCD (Specific Aim 3). In addition, using this derived diffraction correction factor with the PCD measurements made in Chapter 2 and the PCD calibration conducted in Chapter 3, the acoustic power radiated by cavitation over the skin surface during sonophoresis was characterized. This approach enabled comparisons among measurements made for IFS and HFS to be made in order to identify the relationships between the power radiated from stable cavitation over the
skin surface to changes in skin resistance, independent of the insonation frequency. In addition to sonophoresis, this approach provides fully system-independent and quantitative values that directly characterize cavitation and may be used as a standard metric for cavitation emission measurements for other cavitation-based therapies.

Finally, Chapter 5 summarizes the findings of this dissertation and potential future studies are discussed. Specifically, based on the key findings of this dissertation, the implications for future sonophoresis treatment design are discerned. In addition, the potential for expanding the calibration and corrected emission measurements techniques developed here to other cavitation-based therapies is elaborated.
Chapter 2

Sonophoresis: ultrasound-enhanced skin permeability

2.1 Introduction

Although cavitation has been widely accepted as the primary enhancement mechanism of sonophoresis, the effects on skin permeability due to the various location(s) and type(s) of cavitation that occur during IFS and HFS have not been fully elucidated. These mechanisms are likely different from LFS since higher ultrasound frequencies result in resonant sized bubbles with smaller dimensions and different dynamics than those produced by lower frequencies [Yang et al., 2004]. The conclusions of previous IFS and HFS investigations have been based on theoretically derived results or indirect observations and have yet to be investigated systematically and quantitatively [Mitragotri et al., 1995b, Ueda et al., 2009]. This lack of mechanistic understanding has therefore inhibited optimization efforts to exploit and control specific permeability-
enhancing mechanisms of cavitation necessary to improve treatment efficacy of IFS and HFS. Moreover, although sonophoresis using higher frequency ultrasound has a long track record of safety [Polat et al., 2011], the reversibility and longevity of enhanced permeability due to IFS and HFS is not well characterized.

Here, mechanisms of cavitation were quantitatively investigated by passively measuring acoustic emissions associated with specific cavitation activity isolated within or outside porcine skin during \textit{in vitro} IFS and HFS experiments. Since the correlation between the electrical impedance and permeability of skin has been quantitatively defined for a variety of compounds, including hydrophilic and hydrophobic solutes [Karande et al., 2006, Tezel et al., 2003, Tang et al., 2001], skin resistance can be used to instantaneously monitor alterations made to the skin barrier during sonophoresis as a surrogate measure of permeability [Mitragotri et al., 1995b, Tang et al., 2002b, Tezel et al., 2002] and to monitor the barrier recovery [Curdy et al., 2002, Cancel et al., 2004, Gupta and Prausnitz, 2009]. Resistance measurements of porcine skin were made to quantify time-dependent changes of skin permeability and subsequent recovery during and after sonophoresis. Measured emission levels and skin resistance values were compared to clarify the potential roles of cavitation during IFS and HFS treatments, including (1) permeabilization associated with cavitation activity inside or outside the skin, (2) the relationship of emissions from distinct cavitation type(s) with treatment efficacy metrics, such as faster and greater overall permeabilization of skin, and (3) the reversibility and longevity of perturbations made to the skin due to ultrasound treatments in these frequency regimes.
2.2 Materials and Methods

2.2.1 Skin Tissue Preparation

Fresh skin was harvested from the front lateral flank of female Yorkshire X swine immediately post mortem under the approval of the Institutional Animal Care and Use Committee (IACUC) of the University of Cincinnati. Full thickness skin (FTS) samples were prepared by removing subcutaneous tissue and excess hair, sectioned into 6×6 cm square pieces, and stored at −80 °C until use within a period of less than 3 months, to avoid altering the barrier due to storage [Harrison et al., 1984, Kasting and Bowman, 1990]. Experiments were conducted using in vitro porcine skin since previous studies have shown, utilizing skin electrical resistance as an indicator of skin permeabilization, that porcine skin may provide similar drug transport pathways as human skin in vitro [Tang et al., 2001] and that in vivo permeability may be accurately predicted from in vitro measurements of porcine skin [Tang et al., 2002a]. Additionally, porcine skin was used due to its comparable histological, biochemical, and in vitro permeability characteristics to human skin [Barbero and Frasch, 2009, Godin and Touitou, 2007].

Prior to use, FTS samples were thawed and hydrated in 0.01 M phosphate buffered saline (PBS; Sigma-Aldrich, St. Louis, MO USA) at 4 °C for 20-24 hours to eliminate temporal changes of skin permeability due to a hydration gradient during experiments [Behl et al., 1980]. The gas content of PBS used for FTS hydration and experiments was monitored using a meter (WD-35641, Oakton Instruments, Vernon Hills, IL USA) to measure dissolved oxygen (DO) as
a surrogate measure of total gas saturation. The DO content of skin was controlled by hydrating skin using PBS that was either: (1) degassed by placing under vacuum while in a sonication bath for approximately one hour to lower the DO below 20%, or (2) allowed to saturate in open air, permitting the gas content to reach a DO of at least 80%. By controlling the DO content of skin samples, the location of cavitation could be isolated and the location of permeability enhancing cavitation could be assessed directly. After hydration and immediately prior to use, skin was acclimated to room temperature for 30 minutes in fresh PBS with approximately the same DO content as used during hydration to eliminate effects from tissue heating [Park et al., 2008].

2.2.2 Ultrasound Apparatus and In Vitro Experiments

Hydrated FTS samples were trimmed into circular sections 4.5 cm in diameter and placed in a custom made vertical diffusion cell. The diffusion cell is a vertically oriented apparatus comprised of a PBS filled donor and receiver compartment, representing the external and subcutaneous environment to the FTS sample, respectively, shown schematically in Figure 2.1. The skin surface was placed in the apparatus with the stratum corneum facing the donor compartment. For all trials, degassed PBS was used to fill the receiver compartment to eliminate cavitation activity posterior to FTS. The following combinations of gas-content controlled donor PBS and skin were employed to isolate the location of cavitation during IFS and HFS. First, a series of experiments were conducted in the absence of ultrasound exposure to serve as sham trials. Second, to suppress all cavitation
activity, degassed FTS was used in combination with degassed PBS in the donor compartment, experiments referred to hereafter as ‘controls.’ Third, to isolate cavitation activity to the donor PBS only, outside the skin, degassed FTS was used in combination with air saturated donor PBS, experiments referred to hereafter as ‘skin degassed.’ Fourth, to isolate cavitation activity within the skin only, FTS hydrated in air-saturated PBS was used in combination with degassed donor PBS, experiments referred to hereafter as ‘PBS degassed.’

All experimental equipment and data acquisitions used during sonophoresis, illustrated in Figure 2.1, were controlled by a custom MATLAB (R2012a, The Mathworks, Inc., Natick, MA USA) script used to connect and sync a PC to a digital multimeter (34401A, Agilent, Santa Clara, CA USA), an arbitrary waveform generator (33220A, Agilent, Santa Clara, CA USA), and a custom microcontroller circuit used to power a 50 Ohm electro-mechanical, radio-frequency switch (50S-1313+12-SMA, JFW Industries, Inc., Indianapolis, IN USA). Ultrasound was produced by sending a sinusoidal signal from the generator (Agilent, Santa Clara, Ca, USA) through the switch to a radio-frequency amplifier (3100L, ENI, Mountain View, CA USA) to power one of two source transducers operated at center frequencies of 0.41 and 2.0 MHz (IX-887 and IX-887, UTX, Ithaca, NY USA) as representative frequencies of IFS and HFS, respectively. The transducer was placed 1.1 and 3.1 cm from the skin surface during IFS and HFS, respectively. Skin was treated for 30 minutes with ultrasound that was generated in a pulsed continuous-wave mode with a 20% duty cycle (1 second on, 4 seconds off) for either insonation frequency.
Figure 2.1: Schematic of experimental apparatus. Shown is the electronic instrument configuration used for sonophoresis, measurement of skin resistance, and passive acquisition of acoustic emissions from cavitation activity throughout treatment. *In vitro* porcine skin was used to separate the donor and receiver compartments of a custom made diffusion cell that was designed to accommodate the treatment transducer and a passive cavitation detector in the donor and receiver compartments, respectively, and two electrodes placed on either side of the skin in-series with a known resistor.

Each transducer was calibrated by measuring the acoustic power output (APO) using a radiation force balance (UPM-DT-10E, Ohmic Instruments, Easton, MD USA) and the peak rarefractional pressure at the skin surface using calibrated hydrophones. The APO generated by the 0.41 MHz transducer for IFS trials was 0.79 and 1.68 W. Corresponding peak negative pressures measured by a
calibrated hydrophone (TC 4038, Teledyne Reson, Goleta, CA USA) for IFS trials were 282 and 404 kPa. The APO generated by the 2.0 MHz transducer for HFS trials was 8.44 and 21.7 W. Corresponding peak rarefactional pressures measured by a calibrated hydrophone (1239, Precision Acoustics, Dorchester, Dorset, UK) for HFS trials was 0.53 and 0.77 MPa.

The insonation parameters used for IFS and HFS were chosen based on the following criteria: (1) the lower APO used for each frequency was sufficient to elicit consistent subharmonic emissions from cavitation occurring in air saturated in the diffusion cell without skin, (2) the higher APO applied for each frequency was sufficient to elicit occasional broadband emissions from inertial cavitation occurring within air saturated water in the diffusion cell without skin, and (3) the pulse length and duty cycle used in combination with the chosen APOs for each frequency limited tissue heating at the skin surface to no greater than a 5 °C increase above room temperature for any trial. To reduce effects from reflections or standing waves, sound absorbing rubber (Aptflex F28, Precision Acoustics LTD, Dorchester, Dorset, UK), with an acoustic impedance similar to water and a measured reflection coefficient less than −20 dB throughout the investigated frequency range, was placed at the bottom of the receiver compartment perpendicular to the propagating acoustic wave from the source transducer.
2.2.3 Passive Cavitation Detection and Analysis

Acoustic emissions arising from cavitation within the skin and surrounding medium during sonophoresis were detected by a 1-MHz, 25 mm circular diameter, unfocused, broadband transducer (C302, Panametrics, Waltham, MA USA) employed as a receive-only, passive cavitation detector (PCD), similar to methods used by others [Mast et al., 2008b]. The PCD was mounted on the sidewall of the receiver compartment facing the skin sample, approximately 45 mm from the center of the FTS sample. This off-center configuration was used to reduce direct signals from the insonation transducer, therefore increasing the dynamic range available for cavitation detection.

Raw time-domain signals received by the PCD were passed through a 1:1 50 Ohm isolation transformer (M0203, TTE Inc., Los Angeles, CA USA), used to remove the PCD as a ground source during resistance measurements, and sent to a preamplifier (SR560, Stanford Research Systems, Sunnyvale, CA USA). Audible, fundamental, and higher harmonic contributions to the received signals were reduced by the preamplifier using a 6 dB per octave band-pass filter with a passband of 10-300 kHz during IFS and 10-1000 kHz during HFS experiments. Filtered signals were then amplified by a factor of 100 during IFS and by 500 during HFS by the preamplifier. For each 1-second insonation pulse during sonophoresis, an oscilloscope (Waverunner 6050A, LeCroy, Chestnut Ridge, NY USA) was triggered to synchronously digitize and record three successive filtered and amplified PCD-received signals of 200 ms duration, with a 100 ms delay.
between acquisitions, at 10 MHz sampling frequency, and vertical resolution of 8 bits. Additionally, ten consecutive PCD signals were acquired immediately prior to each experiment to serve as a reference signal and indicate the electronic noise floor.

A custom MATLAB algorithm was used to estimate power spectra of each acquired time-domain signal by using the method of averaged periodograms [Welch, 1967]. Briefly, each 200 ms PCD signal was segmented by non-overlapping 1 ms rectangular windows, the discrete Fourier transform of each segment was computed, and the squared magnitude of the individual segments were averaged to acquire power spectra with a frequency resolution of 1 kHz. Spectral energy in the frequency band at half of the center insonation frequency was extracted from each power spectrum as an indicator of subharmonic emissions from stable cavitation, while spectral energy in the band 0.25–0.35 MHz was extracted to characterize broadband emissions arising from inertial cavitation. Using the same analysis to determine the frequency content of the spectra acquired prior to treatment, the reference noise level was computed to account for background electronic noise and interference artifacts residing within each investigated frequency band. Time-averaged signal-to-noise ratios (SNR) of the subharmonic and broadband frequency bands were calculated as dB-scaled ratios of integrated spectral energy of each investigated frequency band signal, measured during the initial 3 and 30 minutes of treatment, relative to the noise level of the reference signal measurement over the corresponding frequency bands and time duration.
Skin electrical resistance was measured during sonophoresis for all trials to serve as an instantaneous and surrogate measure of skin permeability, where increased permeability is indicated by decreased resistance [Karande et al., 2006, Tezel et al., 2003, Tang et al., 2001]. Throughout each experiment the function generator was triggered to supply a short-duration (1–2 seconds), 100 mV$_{RMS}$, 10 Hz AC voltage through the electro-mechanical switch to a circuit consisting of a pair of 4 mm Ag/AgCl electrodes (E242, In Vivo Metric, Healdsburg, CA USA), placed in the donor and receiver compartments 1.5 cm from the skin sample, and an in-series 1 kΩ resistor (measured prior to each trial as 1001.5 ± 11.8 Ω). A multimeter was synchronously triggered to measure the potential across the known in-series resistor. The electrical resistance of skin was then calculated as a function of time using Ohm’s Law by

$$R_{\text{skin}}(t) = \left( \frac{V_{\text{out}}}{V_r} - 1 \right) R_k - R_{f\text{gen}} - R_{\text{PBS}},$$

(2.1)

where $R_{\text{skin}}(t)$ is the time-dependent measure of skin resistance, $V_r$ the potential across the known resistor measured as a function of time, $V_{\text{out}}$ the supplied voltage by the function generator (100 mV$_{RMS}$), $R_k$ the measured resistance value of the known resistor, $R_{f\text{gen}}$ the coupling impedance of the function generator (50 Ω), and $R_{\text{PBS}}$ the resistance of the 3.0 cm column of PBS separating the electrodes, measured prior to experiments in the absence of skin. Time-dependent skin resistance measurements were made immediately prior to ultrasound exposure and
at 10 seconds intervals thereafter, during every second quiescent period of pulsed ultrasound. Post-treatment skin resistance measurements were made at 30 second intervals for 30 minutes after treatment had ceased. All time-dependent resistance measurements were then normalized by the initial skin resistance measured prior to treatment. Additionally, prior to each experiment the resistivity of skin was calculated by multiplying the initially measured resistance value by the skin surface area exposed to PBS. To ensure that the skin barrier was fully intact after harvesting and freezing, skin samples with a resistivity under 35 kΩ · cm² were discarded [Kasting and Bowman, 1990].

The exponential decrease of normalized skin resistance as a function of time during each individual treatment was approximated by a least-squares fit to the model

$$\frac{R(t)}{R_0} = 1 - A(1 - e^{-\kappa t}),$$

where $R(t)$ is the skin resistance as a function of time (ohms), $R_0$ the initial skin resistance value (ohms), $A$ the normalized skin resistance coefficient (dimensionless), $\kappa$ the exponential decay rate constant (min⁻¹), and $t$ the elapsed time (min). The exponential regression was applied to the time-dependent normalized skin resistance data of each individual trial and a coefficient of determination was calculated to identity the goodness of fit. From Equation 2.2, the initial rate ($IR$) of normalized skin resistance decrease was then linearly
approximated as

$$IR = -A\kappa.$$  \hspace{1cm} (2.3)

Using the measured resistance value of skin at the end of treatment ($t = 30$), the total relative decrease in skin resistance due to sonophoresis ($DR$) was characterized by

$$DR = 1 - R(30)/R_0.$$  \hspace{1cm} (2.4)

Post-treatment skin resistance data was first analyzed by applying a linear regression to the normalized skin resistance and a coefficient of determination was calculated to identify the goodness of fit to each individual trial. Second, the slope of the least-squares fit was determined to characterize the post-treatment rate of normalized skin resistance recovery ($dR/dt$). Finally, using the calculated rate of skin resistance recovery ($dR/dt$) and the calculated $DR$ value from Equation 2.4, the post-treatment normalized skin resistance was extrapolated to estimate the number of hours required for skin to fully recover to its initial value ($\tau$) as

$$\tau = \frac{DR}{dR/dt}.$$  \hspace{1cm} (2.5)
2.2.5 Statistical Analysis

Group means were compared for measured acoustic emission levels, initial rate of resistance decrease ($IR$), relative resistance decrease after treatment ($DR$), and post-treatment resistance recovery rate. Grouped data was analyzed first using the Shapiro-Wilk test to check normality ($p \geq 0.05$) of each variable set. Significant ($p < 0.05$) differences among group means and medians of normally distributed variables were identified by one-way analysis of variance (ANOVA) and non-normally distributed variables by Kruskal–Wallis ANOVA (KWANOVA), respectively. Third, post-hoc pairwise comparisons were made to identify significant ($p < 0.05$) differences between group means and medians using Tukey’s method for variables analyzed by ANOVA and the Holm-Bonferroni method for variables analyzed by KWANOVA, respectively.

Correlation analyses were conducted among corresponding time-dependent emission levels and characteristic skin resistance reduction values obtained during IFS and HFS. A Hotelling-Williams $t$-test was used to delineate significant differences among correlation coefficients of dependent variables. Significance of correlation coefficients and corresponding $t$-test were considered at a significance level of $\alpha = 0.05$ (two-tailed). All statistical analyses were conducted using R software (version 3.0.2) [R Core Team, 2013].
Figure 2.2: Representative analysis of passively detected acoustic emissions from cavitation. (a) Two representative frequency spectra of acoustic emissions obtained during sonophoresis ($f_0=2.0$ MHz). Frequency content consistent with cavitation is highlighted at the subharmonic ($f_0/2$) band in both spectra and by a rise in broadband noise in the red spectrum. Representative time histories of (b) subharmonic and (c) broadband frequency band signal-to-noise ratios (SNR).

2.3 Results

2.3.1 Acoustic emissions during IFS and HFS

Shown in Figure 2.2(a) are two representative PCD spectra measured during HFS. Frequency content within each spectrum consists of a strong signal in the fundamental frequency band ($f_0$, 2.0 MHz) and a rise in frequency content indicative of cavitation activity such as subharmonics ($f_0/2$) and the manifestation of an increased broadband noise level. Spectra such as these, measured throughout each trial, were used to quantify stable and inertial cavitation activity by calculating time-dependent SNR of the subharmonic and broadband frequency bands as shown, respectively, in Figures 2.2(b) and (c).

Mean values of time-averaged subharmonic and broadband emission levels measured for each group treated with IFS (0.41 MHz), shown in Figures 2.3(a) and (b), respectively, were found to be significantly different by ANOVA ($p = 6.62 \times$
Figure 2.3: Time-averaged signal-to-noise ratios (SNR) of (a) subharmonic and (b) broadband emissions acquired during 30-minute intermediate-frequency sonophoresis (IFS) experiments. Results are mean SNR values measured during trials that suppressed all cavitation (control), isolated cavitation activity to within the skin only (PBS degassed), and isolated cavitation activity outside of skin only (skin degassed) during IFS employing acoustic powers of 0.79 and 1.68 W. Error bars indicate one standard deviation and groups that produced a significantly greater SNR than control groups are indicated (⋆).

$10^{-8}$ and $p = 2.25 \times 10^{-3}$, respectively). Multiple comparisons among the means of the various treatment groups revealed that the near zero-dB emission levels measured during IFS trials that suppressed all cavitation activity (control) were not significantly different than those measured during any of the trials that isolated cavitation activity to within the skin only (PBS degassed) for subharmonic and broadband emission levels ($p > 0.88$ and $p > 0.95$, respectively). In comparison to the PBS-degassed and control groups, IFS trials that isolated cavitation activity to the donor PBS outside the skin (skin degassed) produced significantly greater subharmonic emission levels for all employed APOs ($p < 10^{-5}$). Among each
Figure 2.4: Time-averaged signal-to-noise ratios (SNR) of (a) subharmonic and (b) broadband emissions acquired during 30-minute high-frequency sonophoresis (HFS) experiments. Results are mean SNR values for trials that suppressed all cavitation (control), isolated cavitation activity to within the skin only (PBS degassed), and isolated cavitation activity outside of skin only (skin degassed) during HFS employing acoustic powers of 8.44 and 21.7 W. Error bars indicate one standard deviation and groups that produced a significantly greater SNR than the control groups are indicated (⋆).

Individual treatment group pair, no significant increase of the subharmonic or broadband SNR was measured when the APO was increased from 0.79 to 1.68 W. However, in comparison to the PBS-degassed and control trials, the broadband emission level was only significantly greater \( (p < 0.01) \) for the higher 1.68 W APO.

Mean values of time-averaged subharmonic and broadband emission levels measured for each group treated with HFS (2.0 MHz), shown in Figures 2.4(a) and (b), respectively, were found to be significantly different by ANOVA \( (p = 1.23 \times 10^{-8} \text{ and } p = 7.44 \times 10^{-4}, \text{ respectively}) \). Multiple comparisons among the means of the various treatment groups revealed that in comparison to the
near zero-dB emission levels of the control trials, all of the PBS-degassed and skin-degassed trials produced significantly greater subharmonic emission levels ($p < 10^{-4}$). Among the skin-degassed and PBS-degassed trials, when either APO was employed, the subharmonic emission levels were not significantly different ($p > 0.35$). Additionally, among each treatment group pair, no significant increase of the subharmonic SNR was measured when the APO was increased from 8.44 to 21.7 W ($p > 0.34$). However, broadband emission levels measured during skin-degassed and PBS-degassed trials were significantly greater than the control trials only when the higher 21.7 W APO was employed ($p < 0.025$).

### 2.3.2 Reduction of skin resistance during IFS and HFS

Normalized skin resistance, averaged for each treatment group, is shown as a function of time in Figures 2.5(a) and (b) during (‘ultrasound on’) 30-minute IFS and HFS treatments, respectively. The time-dependent reduction of skin resistance during sonophoresis, indicating an increase in skin permeability, is shown in these plots to be approximately exponential during the 30-minute treatments, which is reflected by the high values calculated for the coefficients of determination for the fit to Equation 2 ($r^2 = 0.908\pm0.097$ mean and standard deviation of all trials). In Figures 2.6(a) and (b) the mean and standard deviation of the initial rate ($IR$) and total relative reduction ($DR$) of skin resistance, respectively, are shown for all trials; both were found to be significantly different among treatment groups by KWANOVA ($p = 1.62 \times 10^{-7}$ and $p = 4.57 \times 10^{-8}$, respectively).
Figure 2.5: Normalized skin resistance as a function of time during (‘ultrasound on’) and after (‘ultrasound off’) for (a) intermediate- (IFS) and (b) high-frequency sonophoresis (HFS) trials. Time-dependent values are averaged for groups that suppressed all cavitation (control), isolated cavitation activity within the skin only (PBS degassed), and isolated cavitation activity outside of skin (skin degassed) for each respective acoustic power employed during IFS and HFS. Error bars indicate the value of one standard deviation at representative time points during and after treatment.
Multiple comparisons among the mean $IR$ or $DR$ for groups treated with IFS revealed no significant differences among any of the PBS-degassed, control, and trials that received no ultrasound exposure (sham) ($p > 0.75$). However, in comparison to these trials, significantly greater $IR$ and $DR$ values were measured during IFS skin-degassed trials when either APO was employed ($p < 10^{-3}$ and $p < 10^{-4}$, respectively). Normalized skin resistance during the skin-degassed trials initially decreased with $IR$ rates of $8.71\pm6.50$ and $11.9\pm5.10$ min$^{-1}$ and decreased overall with $DR$ values of $44.3\%\pm18.8\%$ and $46.3\%\pm6.24\%$ at the end of treatment, respectively with increasing APO. No significant difference in either $IR$ or $DR$ was observed when the APO was increased among any individual group pair treated with IFS.

Additionally shown in Figures 2.6(a) and (b) are the mean and standard deviation of $IR$ and $DR$ values, respectively, measured for each HFS trial. Unlike the results of the IFS trials, the HFS control group employing either APO produced significantly greater $IR$ and $DR$ values than the sham trials ($p < 0.05$). However, the $IR$ and $DR$ values measured for the sham and HFS control groups were both significantly lower than for the HFS skin-degassed and PBS-degassed groups treated with either APO ($p < 0.05$). Skin resistance during the skin-degassed trials reduced initially at rates of $3.90\pm2.60$ and $6.50\pm2.61$ min$^{-1}$ and decreased overall by $26.58\%\pm12.36\%$ and $34.20\%\pm10.21\%$ at the end of treatment, respectively with increasing APO. During the PBS-degassed trials, skin resistance initially reduced at rates of $4.35\pm2.11$ and $6.52\pm3.11$ min$^{-1}$ and decreased overall by $28.49\%\pm6.85\%$ and $43.10\%\pm11.52\%$ at the end of treatment, respectively with increasing APO.
Figure 2.6: Mean values of (a) initial decay rate (IR) and (b) end of treatment reduction (DR) of normalized skin resistance among trials treated with no ultrasound (sham) and intermediate- (IFS) or high-frequency sonophoresis (HFS) that suppressed all cavitation (control), isolated cavitation activity to within the skin only (PBS degassed), or isolated cavitation activity outside of skin (skin degassed). Error bars indicate one standard deviation. Groups that produced significantly greater values than the sham group (×) and groups that produced significantly greater values than both the sham and respective control trials (*) are indicated.
All treatment groups that produced significant acoustic emissions (Figures 3 and 4), in comparison to the control trials, also produced significantly greater $IR$ and $DR$ values (Figure 6) than trials that did not produce significant acoustic emissions. The $IR$ and $DR$ values obtained during the IFS skin-degassed, HFS skin-degassed, and HFS PBS-degassed trials were significantly greater than those obtained during the IFS control, IFS PBS-degassed, and HFS control trials ($p < 0.05$). No significant difference in either $IR$ or $DR$ was observed when the APO was increased among any individual group pair treated with HFS and no significant difference was found when comparing the $IR$ or $DR$ values between the HFS skin-degassed and PBS-degassed groups. The $IR$ and $DR$ values were not significantly different among the IFS skin-degassed, HFS skin-degassed and PBS-degassed groups ($p > 0.99$), all trials that produced significant acoustic emissions. Likewise, $IR$ and $DR$ values were not significantly different among the IFS PBS-degassed, IFS control, and HFS control groups ($p > 0.99$), all trials that did not produce significant acoustic emissions from cavitation.

### 2.3.3 Correlation among acoustic emissions and changes in skin resistance

Calculated correlation coefficients between skin resistance reductions, characterized by $IR$ and $DR$, and corresponding time-averaged subharmonic and broadband emission levels of all 30 IFS trials are summarized in Table 2.1. The significant correlations of $IR$ with subharmonic ($r = 0.899, p = 1.38 \times 10^{-11}$) and broadband ($r = 0.816, p = 3.84 \times 10^{-8}$) emission levels, measured during the
Figure 2.7: IFS results: Initial decay rate (IR) and end of treatment reduction (DR) of normalized skin resistance vs. time-averaged subharmonic (a, c) and broadband (b, d) signal-to-noise ratios (SNR) measured over 3 and 30 minutes, respectively, during intermediate-frequency sonophoresis (IFS). Points are shown for all IFS trials (N = 30). The dashed line indicates the linear regression line for all points.
Table 2.1: Correlation matrix for initial rate of skin resistance decrease (IR) and the total relative skin resistance decrease at the end of treatment (DR) with the time-averaged signal-to-noise ratio (SNR) of each investigated frequency band measured over the initial 3 and 30 minutes, respectively, during IFS (0.41 MHz) treatment.

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<td>t-statistic</td>
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Boldfaced correlation coefficient and t-statistic values indicate statistical significance ($p < 0.05$).

initial 3 minutes of treatment, are illustrated in Figures 2.7(a) and (b), respectively. A significant correlation was found between subharmonic and broadband SNR ($r = 0.777$, $p = 1.67 \times 10^{-7}$) and no significant difference was found between correlation coefficients of IR with either the subharmonic or broadband SNR ($t = 1.62$, $p = 0.12$).

Scatter plots shown in Figures 2.7(c) and (d) illustrate the significant correlations between DR with subharmonic ($r = 0.928$, $p = 1.67 \times 10^{-13}$) and broadband ($r = 0.633$, $p = 1.74 \times 10^{-4}$) emission levels, respectively, measured over the duration of each 30-minute IFS treatment. Despite a significant correlation between the two measured emission levels ($r = 0.777$, $p = 4.32 \times 10^{-7}$), DR correlated significantly greater with the subharmonic than with the broadband SNR ($t = 6.42$, $p = 5.9 \times 10^{-7}$).

Calculated correlation coefficients between the skin resistance decrease characteristics IR and DR with corresponding time-averaged subharmonic and broadband emission levels of all 34 HFS trials are summarized in Table 2.2. The
significant correlation of \( IR \) with subharmonic \( (r = 0.896, p = 8.01 \times 10^{-13}) \) and broadband \( (r = 0.528, p = 1.30 \times 10^{-3}) \) emission levels, measured during the initial 3 minutes of treatment, are illustrated in Figures 2.8(a) and (b), respectively. Despite the significant correlation between the two measured emission types \( (r = 0.731, p = 9.01 \times 10^{-7}) \), \( IR \) correlated significantly greater with the subharmonic than with the broadband SNR \( (t = 6.74, p = 1.30 \times 10^{-7}) \).

Table 2.2: Correlation matrix for initial rate of skin resistance decrease \( (IR) \) and the total relative skin resistance decrease at the end of treatment \( (DR) \) with the time-averaged signal-to-noise ratio (SNR) of each investigated frequency band measured over the initial 3 and 30 minutes, respectively, during HFS (2.0 MHz) treatment.

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<td>Broadband</td>
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<td>Broadband</td>
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<td>( t )-statistic</td>
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<td>Broadband</td>
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<td>( t )-statistic</td>
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Boldfaced correlation coefficient and \( t \)-statistic values indicate statistical significance \( (p < 0.05) \).

Scatter plots shown in Figures 2.8(c) and (d) illustrate the significant correlations of \( DR \) with subharmonic \( (r = 0.888, p = 2.42 \times 10^{-12}) \) and broadband \( (r = 0.613, p = 1.16 \times 10^{-4}) \) emission levels, respectively, measured over the duration of each 30-minute HFS treatment. \( DR \) correlated significantly greater with the subharmonic than with the broadband SNR \( (t = 4.25, p = 1.70 \times 10^{-7}) \), despite a strong correlation between the measured SNR of the two emission types \( (r = 0.709, p = 2.67 \times 10^{-6}) \).
Figure 2.8: HFS results: Initial decay rate (IR) and end of treatment reduction (DR) of normalized skin resistance vs. time-averaged subharmonic (a, c) and broadband (b, d) signal-to-noise ratios (SNR) measured over 3 and 30 minutes, respectively, during high-frequency sonophoresis (HFS). Points are shown for all HFS trials (N = 34). The dashed line indicates the linear regression line for all points.
2.3.4 Skin resistance recovery after IFS and HFS

Normalized skin resistance measurements made over 30 minutes immediately after IFS and HFS treatment (‘ultrasound off’) is shown as a function of time for the mean of each treatment group in Figures 2.5(a) and (b), respectively. Considering the approximate linear increase of skin resistance with time after treatment, a linear regression, characterized by high coefficients of determination ($r^2 = 0.960 \pm 0.064$ mean and standard deviation of all trials), was applied to each trial and the slope of the fit was used to quantify the rate of skin resistance recovery. The mean rate of recovery of the IFS skin-degassed, HFS skin-degassed and PBS-degassed groups, trials that incurred significant resistance reductions during treatment and shown in Figure 2.9(a), were found to be significantly different by ANOVA ($p = 2.6 \times 10^{-3}$). Multiple group comparisons of means revealed that the recovery rates were not significantly different among the PBS-degassed and skin-degassed groups treated with HFS employing either APO. Additionally, the recovery rates among the pair of IFS skin-degassed groups employing either APO were not significantly different. However, in comparison to the HFS trials, the rate of recovery of the IFS groups was significantly slower ($p = 3.75 \times 10^{-5}$).

Considering the approximately linear increase of normalized skin resistance after treatment, the recovery of skin resistance was assumed to continue its linear trajectory beyond the 30-minute measurement period and, using Equation 2.5, the approximate time required for skin resistance to fully recover was estimated. The group means of recovery time for the IFS skin-degassed, HFS skin-degassed and PBS-degassed trials shown in Figure 2.9(b), groups that all incurred significant
Figure 2.9: Post-treatment (a) rate of recovery and (b) estimated time for complete recovery of normalized skin resistance. Average values are shown for trials that isolated cavitation outside of the skin only (skin degassed) during intermediate-frequency sonophoresis (IFS, 0.41 MHz ultrasound) and trials that isolated cavitation inside of the skin only (PBS degassed) or outside of the skin only (skin degassed) during high-frequency sonophoresis (HFS, 2.0 MHz ultrasound). Error bars indicate one standard deviation. Skin resistance recovered significantly faster after HFS than IFS treatment (indicated by ⋆), and the time for skin resistance to fully recover was significantly greater after IFS than HFS treatment (indicated by ×).

Resistance reductions during treatment, were found to be significantly different by ANOVA ($p = 3.54 \times 10^{-6}$).

No significant difference was found among the recovery times calculated for any of the HFS skin-degassed and PBS-degassed trials ($p > 0.75$). The recovery times among the IFS skin-degassed group were not significantly different among trials that employed either APO for treatment ($p > 0.95$). However, in comparison to the IFS skin-degassed groups, the trials treated with HFS required significantly greater time to fully recover ($p = 1.87 \times 10^{-2}$).
Figure 2.10: Scatter plots of the estimated time for complete recovery vs. the end of treatment reduction of normalized skin resistance (DR) for all trials treated with (a) intermediate- (IFS) and (b) high-frequency sonophoresis (HFS). The dashed line indicates the linear regression line for all points.

Scatter plots shown in Figures 2.10(a) and (b) show the estimated time to recover as a function of DR for all IFS and HFS trials, respectively. The approximately linear relationship between the time to recover and DR is represented in the plot by a trend line (dashed lines) for IFS ($r^2 = 0.829$, $p = 2.88 \times 10^{-12}$) and HFS ($r^2 = 0.625$, $p = 2.17 \times 10^{-9}$) trials. The slopes of the linear least-squares fits to this data indicate that for every 10% decrease in skin resistance incurred during IFS, approximately 3.6 hours would be required for skin to recover that amount. In comparison, the slopes of the linear least-squares fits to this data indicate that for every 10% decrease in skin resistance incurred during HFS, approximately 1.2 hours would be required for skin to recover that amount.
2.4 Discussion

2.4.1 The role of cavitation during IFS

Significant time-dependent skin resistance decreases (Figure 2.6) were measured during IFS only when significant cavitation activity was also present (Figure 2.3). Specifically, in comparison to the near-zero dB emission levels measured during trials that suppressed all cavitation (control) during IFS, significant emission levels were only measured when cavitation was isolated outside the skin (skin-degassed). Correspondingly, in comparison to trials that suppressed all cavitation or isolated potential cavitation activity to within the skin only during IFS, skin resistance decreased at a significantly faster initial rate ($IR$) and achieved a significantly greater overall decrease ($DR$) only when cavitation was isolated outside the skin. These results indicate that the permeabilization effect on skin achieved in this study during IFS was primarily due to cavitation activity outside the skin and, using the insonation parameters explored here, cavitation did not occur within the skin during IFS.

Similar to the results shown in the present study for IFS, Tang et al. demonstrated, by isolating the location of cavitation activity, that cavitation does not occur within the skin during LFS and that the permeabilization effect these authors measured, quantified by changes in skin impedance, was solely due to cavitation activity outside of the skin [Tang et al., 2002b]. The observed absence of permeability-enhancing cavitation within skin in this and previous studies during
IFS and LFS is likely due to the physical dimensions of the lacunar regions within the SC, where cavitation could occur, being smaller than the diameter of bubbles that are resonant to the applied frequencies [Simonin, 1995]. In water, for example, the theoretical diameter of free bubbles resonant to 0.41 MHz ultrasound is approximately 7.5 µm, larger than the nominal lumen diameter of sweat ducts (5 µm) [Scheuplein, 1967] and equivalent to the thickness of the SC (10 µm) [Holbrook and Odland, 1974]. Considering also that the threshold to initiate cavitation shifts towards larger bubbles in tissue than in aqueous phase [Yang and Church, 2005], the physical limitations of the skin dimensions to accommodate bubbles resonant to LFS likely also inhibits the presence of bubbles large enough to be excited by IFS. Further, although resonant sized bubbles are not a prerequisite for the onset of ultrasound-induced cavitation, the lack of identifiable emissions or associated skin permeability enhancement in this and previous studies indicates that the dynamic response from sub-resonant sized bubbles, which could exist within the SC, are negligible for cavitation activity occurring within the skin during IFS.

Although the location of cavitation responsible for the permeabilization effect on skin during IFS is shown to be similar to that of LFS, the type of cavitation responsible for enhancement among the two treatment-frequency regimes is shown here to be different. In separate studies, Tang et al. and Tezel et al. demonstrated that enhancement of skin electrical conductivity achieved during LFS correlated more strongly with broadband emissions, from inertial cavitation, than with subharmonic emissions, from stable cavitation [Tang et al., 2002b, Tezel et al., 2002]. In the present study, significantly greater IR and DR values were found for
the IFS skin-degassed trials in comparison to the sham and control when employing either APO for treatment. However, among these trials, only the subharmonic, not broadband, emission levels measured when either APO was employed were found to be significantly greater than the near-zero emission levels of the control trials, suggesting that stable cavitation may play a greater role during IFS than during LFS. This indication is further supported by the significant and greater correlations of both the IR and the DR values with the subharmonic than with the broadband emission levels (Figure 2.7, Table 2.1).

2.4.2 The role of cavitation during HFS

Similar to the IFS results, skin resistance during HFS was significantly reduced only when significant emissions from cavitation were present, confirming that, similar to LFS and IFS, the bilization effect observed during HFS is primarily due to cavitation. However, unlike the IFS trials explored here and the findings of previous LFS studies, significant acoustic emission levels (Figure 2.4) and corresponding time-dependent skin resistance decreases (Figure 2.6) were measured when cavitation was isolated outside of the skin (skin-degassed) as well as when cavitation was isolated within the skin (PBS-degassed). Additionally, comparing corresponding IR and DR values between the skin-degassed and PBS-degassed trials provided no significant differences, indicating that a comparable permeabilization effect on skin can be achieved during HFS by cavitation activity outside the skin as cavitation inside the skin.

Cavitation as a primary mechanism of enhancement during HFS was
suggested by Bommannan et al. [Bommannan et al., 1992] and later investigated mechanistically by others, identifying in separate studies that the permeabilization effect incurred during HFS may be induced by cavitation occurring within the skin only [Mitragotri et al., 1995b] and, more recently, that cavitation activity induced outside the skin may play a contributory role [Park et al., 2012]. Although the mechanistic conclusions made by these previous authors were based on indirect observations, the results shown in the present study quantitatively confirm both conclusions, that significant skin permeabilization during HFS can be achieved in the presence of cavitation activity within as well as outside the skin.

In a recent study by Park et al., transdermal penetration of FITC-dextrans was shown to be significantly increased by HFS when UCAs were used as cavitation nuclei outside of the skin [Park et al., 2012]. These authors concluded that since the acoustic pressures employed were relatively low, the most likely mechanism of enhancement was microstreaming associated with stable cavitation. Although the conclusions made by these authors were based on a theoretical prediction of cavitation activity onset rather than direct observations, skin resistance decreases during HFS were quantitatively shown in the present study to be more strongly related to subharmonic emissions, associated with stable cavitation, than with broadband emissions, associated with inertial cavitation. In comparison to the control and sham trials conducted here, significantly greater $IR$ and $DR$ values were produced during HFS skin-degassed and PBS-degassed trials employing either APO for treatment. Correspondingly, these same trials produced significantly greater subharmonic emission levels than controls when either APO
was employed, but did not produce significant broadband emission levels for all trials, indicating that similar to the IFS trials, stable cavitation may play a greater role than inertial cavitation. Furthermore, this indication is supported by the significantly greater correlations of both $IR$ and $DR$ with subharmonic emissions than with broadband emissions.

2.4.3 Contribution of non-cavitation ultrasound mechanisms

In addition to cavitation, other mechanisms during sonophoresis capable of enhancing penetration across the skin include convection transport, thermal effects, and acoustic streaming [Mitragotri, 2007, Polat et al., 2011]. Among these mechanisms, thermal effects from tissue heating are a prevalent and near unavoidable mechanism that alone can result in increased skin permeability [Mitragotri, 2007]. However, tissue heating can be accompanied by adverse bioeffects within the SC, ranging from lipid-associated conformational alterations to irreversible structural perturbations [Gay et al., 1994], even when skin temperatures are kept below 65 $^\circ$C. To avoid these adverse effects during sonophoresis, ultrasound is commonly applied in a pulsed mode to minimize tissue heating [Tang et al., 2002a, Mitragotri et al., 1995a, Mitragotri et al., 1996, Tang et al., 2002b, Wolloch and Kost, 2010, Terahara et al., 2002].

Although a pulsing regime was employed in the present study to minimize thermal effects, tissue heating was not fully eliminated. In order to distinguish the effects of tissue heating and other non-cavitation mechanisms on skin permeability from the effects of cavitation, control trials were conducted by
suppressing cavitation activity. The skin resistance reductions incurred during IFS (5.2%±5.4%) and HFS (7.2%±1.9%) control trials can therefore be attributed directly to non-cavitation mechanisms including tissue heating. The skin resistance reductions measured in the control trials, when cavitation was suppressed, were significantly less than when cavitation was present during sonophoresis using either investigated frequency regime, further indicating the significant role of cavitation during IFS and HFS. These results are consistent with other studies which have shown that low-grade skin temperature increases, comparable to the 5 °C increases measured here, have a negligible effect on skin permeability during LFS [Tang et al., 2002b, Tezel et al., 2002] and HFS [Levy et al., 1989, Bommannan et al., 1992] in comparison to the effect induced by cavitation. Furthermore, post-treatment normalized skin resistance measured for all control trials began to increase immediately after treatment, indicating that the permeability enhancement due solely to low-grade tissue heating and other non-cavitation mechanisms is reversible.

2.4.4 Assessment of post-treatment skin resistance recovery

Skin resistance measurements made over 30 minutes immediately following sonophoresis in this study were used to characterize the reversibility, longevity, and time-dependent recovery of the skin barrier properties in order to satisfy safety concerns and to identify potential modes of application. The approximately linear increase of skin resistance measured immediately after ultrasound was turned off (Figures 2.5 and 2.9a) indicates that the perturbations made to skin during IFS
and HFS were reversible and non-permanent for all exposure conditions. These results are not dissimilar from the findings of other investigations which have shown that the barrier reductions incurred during LFS are also reversible and non-permanent [Cancel et al., 2004, Gupta and Prausnitz, 2009, Mitragotri et al., 1996].

In addition, these results support the hypothesis that cavitation modifies the SC structure during sonophoresis through physical mechanisms, including increased porosity due to shear forces imposed on the SC lipid bilayers by microstreaming and pore generation due to the impact of microjet formations [Tezel and Mitragotri, 2003, Ueda et al., 2009, Park et al., 2012]. In comparison, these perturbations are less severe than those imposed by removing the SC during microdermabrasion [Andrews et al., 2011], or by using chemical or thermal methods such as enhancing compounds [Karande et al., 2006] and thermal ablation [Lee et al., 2011], respectively, that permanently alter the phase behavior of the SC lipid bilayers. Hence, the recovery of the SC barrier after sonophoresis is likely due to the fluid like mobility of the lipid bilayers, as well as other SC components especially in hydrated skin [Silva et al., 2007], capable of migrating and filling cavitation-induced pores over time. Further, the post-treatment recovery of skin is a major advantage of sonophoresis over other treatment approaches, and this recovery may be assessed in the design of treatments depending on the time duration required for enhanced drug delivery.

The time required for the skin barrier to repair after sonophoresis was shown in the present study to increase in duration as the reduction of resistance achieved
during IFS and HFS increased (Figure 2.10), a phenomenon that has likewise been observed in other studies after LFS treatments. Skin resistance has shown to fully recover or exceed the initial pre-treated value within minutes of treatment being terminated when relatively small resistance reductions (< 10%) due to LFS have been observed [Cancel et al., 2004], while relatively large skin resistance reductions (> 95%) have been shown to recover more slowly, requiring 42 hours for the reduced value to recover by a factor of 10 [Gupta and Prausnitz, 2009]. In other cases, relatively large (> 95%) and moderate (60%) skin resistance reductions incurred during LFS have shown to recover by a factor of 2 and 1.2 times the reduced value after 2 hours [Mitragotri et al., 1996], corresponding to approximate rates of recovery of 2 and 5% per hour, respectively. In comparison, over the range of skin resistance reductions observed in the present study (< 70%), skin resistance recovered at rates of approximately 3 and 8% per hour after IFS and HFS, respectively. One possible reason for differences in recovery rates between IFS and HFS may be due to the larger bubbles induced at lower frequencies which create larger perturbations, requiring a greater time duration for the SC lipid bilayers to migrate and fill.

2.4.5 Sonophoresis treatment implications

Considering the similar locations where permeability-enhancing cavitation occurs among IFS and LFS treatments, future IFS studies may benefit from employing techniques that have previously been used to increase the efficacy of LFS by
increasing the occurrence of cavitation activity outside the skin. These techniques include the addition of cavitation nuclei, such as insoluble porous resins [Terahara et al., 2002], to the coupling medium outside the skin or simultaneous application of high-frequency ultrasound to nucleate small bubbles near the skin surface, which then cavitate in response to the lower-frequency ultrasound used for sonophoresis [Schoellhammer et al., 2012]. For HFS treatments, although cavitation activity within skin contributes to the overall permeabilization, optimizing and controlling cavitation within skin tissue may be challenging due to the sparse preexistence of gas bodies and the spatially variable threshold to initiate cavitation activity in living tissue [Fry et al., 1995]. Instead, since cavitation outside of the skin also plays a role during HFS, promoting cavitation in the coupling medium outside the skin, similar to LFS and IFS applications, may be more suitable due to easier control of cavitation in solution rather than in tissue. This may be accomplished by use of ultrasound contrast agents (UCA) as cavitation nuclei in the medium outside the skin [Park et al., 2010, Park et al., 2012], since UCA encapsulated microbubbles are manufactured within a size distribution resonant to high-frequency ultrasound (approximately 2–8 MHz) [Klibanov, 2002]. Additionally, considering the significant dependence of skin permeability enhancement on the presence of subharmonic-producing cavitation during IFS and HFS, treatment efficacy of both frequency regimes may be further improved by optimizing pulsed-ultrasound exposure parameters, such as modifying the duration of the pulse and quiescent periods, to sustain and maximize stable cavitation activity similar to methods developed to improve the efficacy of ultrasound-enhanced thrombolysis
One potential benefit to treatment safety offered by IFS and HFS, in comparison to LFS, is that significant perturbations to the skin barrier for enhanced drug delivery can be achieved using acoustic pressures that are sufficiently high to elicit subharmonic cavitation in solution, but low enough to avoid significant inertial cavitation activity. This is significant since unintended bioeffects associated with stable cavitation are often less harmful that those of inertial cavitation. For example, stable cavitation is capable of transiently increasing permeability of cell membranes in living tissue, but these perturbations are relatively short-lived and reversible (reversible sonoporation) [Wu and Nyborg, 2008]. On the other hand, the more violent fluid dynamics associated with inertial cavitation can lead to more damaging and long-lasting bioeffects in adjacent tissue and vasculature, such as permanent deformation of cellular membranes (non-repairable sonoporation) [Wu and Nyborg, 2008] or rupture of red blood cells (hemolysis) [Everbach et al., 1997]. In addition to safety concerns during IFS and HFS, inertial cavitation may also be undesirable during treatment since the transient destruction of cavitation nuclei may diminish the potential for sustained stable cavitation, therefore reducing its beneficial, permeabilizing effects on the skin barrier.

The long-lasting, though reversible, effect on skin permeability after ultrasound exposure has been utilized among several recent studies to offer sonophoresis as a pretreatment to drug administration [Tang et al., 2002a, Terahara et al., 2002, Schoellhammer et al., 2012]. Clinically, the pretreatment mode is advantageous
over the simultaneous mode, applying ultrasound with the therapeutic agent simultaneously, since the requirement of the patient to wear a device throughout delivery is eliminated. However, the pretreatment mode is largely reliant on large magnitude barrier perturbation during and after ultrasound exposure, which in comparison to IFS and HFS, has previously only been achieved for LFS applications [Mitragotri et al., 1995a, Ueda et al., 2009]. Utilizing the cavitation mechanisms shown here to achieve a greater permeabilization effect on skin during sonophoresis in future treatments may enable pre-treatment as a viable application mode for IFS and HFS. Future sonophoresis treatments may then be specifically designed to utilize a particular ultrasound frequency regime for treatment based on the time required for skin to remain in a state of high permeability. For example, for treatments that require a state of enhanced skin permeability over a short duration with a more rapid recovery of the skin barrier, such as glucose extraction and insulin delivery [Kost, 2002], the use of HFS may be more appropriate and for applications that require delivery to be maintained over a long time period, therefore requiring a slow barrier recovery, for applications such as hormone delivery [Tezel et al., 2003], the use of IFS or LFS may be more applicable.

In separate comparative in vitro studies, LFS has been shown to decrease skin permeability to a greater degree than both IFS [Ueda et al., 2009] and HFS [Mitragotri et al., 1996]. Nonetheless, the clinical relevance of sonophoresis using higher frequency ultrasound is evident as HFS has been used in far more in vivo human trials than LFS, proving to be a safe and effective method of enhancing the transdermal delivery of over 30 molecules of varying lipophilicities (log $K_{o/w} - 4.75$
to 5.25), and relatively low molecular weight (< 1000 Da) in most cases [Polat et al., 2011]. Considering that the limitations of IFS and HFS identified in previous studies have all been observed in experiments with ultrasound exposure and other treatment conditions not specifically optimized to elicit cavitation activity, greater permeabilization may be achieved by specifically designing treatment conditions to exploit cavitation. For example, Park et al. demonstrated in a recent set of *in vivo* rat experiments that in comparison to traditional exposure conditions, not optimized to elicit cavitation, the transdermal penetration of relatively high molecular weight (up to 150 kDa) FITC-dextrans can be significantly increased by HFS when the medium outside of the skin is seeded with cavitation nuclei in the form of UCAs [Park et al., 2012]. Additionally, considering that skin impedance quantitatively relates to the permeability of skin for a variety of permeants, ranging in molecular weight (180–70,000 kDa) and lipophilicity (log $K_{o/w} − 3$ to 4.13), and the distinct relationship between skin resistance reductions and specific cavitation activity elucidated here for IFS and HFS, suggests that future treatments designed to maximize and sustain these permeability-enhancing mechanisms may broaden the range of compounds that can be delivered across the skin.
Chapter 3

Characterizing the receive sensitivity of single-element transducers

3.1 Introduction

The spectral contents of a PCD-measured waveform are typically analyzed to quantify specific bubble activity by temporally integrating the amplitude [Chen et al., 2003c], squared amplitude [Hoerig et al., 2014], or decibel-scaled level [Hallow et al., 2006, Mast et al., 2008b] of the received signal within distinct frequency bands associated with cavitation. However, these are system-dependent measurements, influenced by the frequency response of the receiving electronics and PCD, leading to challenges in comparing results obtained using different measurement configurations. One approach to making direct, system-independent comparisons of cavitation emissions is to make absolute pressure measurements. However, this requires a PCD and receiving system of known sensitivity.

The frequency-dependent receive sensitivity of an PCD can generally be defined

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where \( X(f) \) is the open-circuit voltage generated in response to receiving \( \bar{P}(f) \), the average complex pressure incident over the transducer element, measured in the free field at a given distance from a source [Carstensen, 1947]. Although a number of methods are available for calibrating the receive sensitivity of small needle-type hydrophones [Ludwig and Brendel, 1988, Smith and Bacon, 1990, Labuda et al., 2004], techniques are not well established for calibrating larger and non-flat aperture transducers commonly employed as PCDs.

In addition to the sensitivity of the receiver, the voltage that is acquired and used for analysis may also be influenced by additional signal filtering, gain, or noise due to the system electronics. These additional modifications to the voltage signal can be accounted for within a frequency-dependent factor, \( G_{sys}(f) \). The total calibration factor of the receiving system, \( M_{sys}(f) \), is then the product of the receiver sensitivity, \( M_R(f) \), and frequency response of the electronics, \( G_{sys}(f) \). Using these values in combination with Equation 3.1, the average incident pressure over the surface of a PCD is related to the system-acquired voltage, \( X_{sys}(f) \), by

\[
\bar{P}_R(f) = \frac{X_{sys}(f)}{M_{sys}(f)} = \frac{X_{sys}(f)}{M_R(f) G_{sys}(f)}.
\] (3.2)

Hence, in order to quantify the PCD-received pressure from the system measured voltage, the factors \( G_{sys}(f) \) and \( M_R(f) \) must be characterized.

Quantifying the contribution of the system electronics, \( G_{sys}(f) \), to the system
measured signal is relatively straightforward. However, quantifying the receive sensitivity, $M_R(f)$, following Equations 3.1 and 3.2 is challenging because the average incident pressure, $\bar{P}_R(f)$, over the PCD is subjected to diffraction effects for larger diameter transducers commonly used as a PCD. In addition, the needle-type hydrophones commonly employed for reference pressure measurements have surfaces that are significantly smaller than that of the PCD being calibrated. Hence, using hydrophone-measured pressures as a reference measurement to calibrate a PCD is challenging, especially for calibrating transducers with curved surfaces. These challenges could be averted by using self-reciprocity methods which account for diffraction effects by using a calculated compensation factor [Carstensen, 1947, Zhang et al., 2016]. However, this approach may not be applicable for accurate and broadband calibrations of various receiver geometries due to variations in the spatial properties of the respectively generated acoustic fields, making it challenging to account for calibration uncertainty. Therefore, improved techniques for broadband calibration of receivers using acoustic measurements alone are needed, and the accuracy of these methods need to be assessed.

Acoustic calibration measurements are most challenging for receivers with non-flat surfaces because of challenges in precisely tracing the region in the free field space representing their surface with a calibrated needle-type hydrophone. This challenge is significant because geometrically focused receivers, which have non-flat surfaces, are commonly employed as a PCD due to their higher spatial specificity in comparison to flat, unfocused receivers. For example, these receivers are often used to acquire acoustic emissions from cavitation when the bubble activity and
associated bioeffects are known to be confined to a specific region, such as for bubble dynamics studies [Collin and Coussios, 2011], focused ultrasound treatments [Thomas et al., 2005, Tung et al., 2011], and lithotripsy [Cleveland et al., 2000]. In comparison, unfocused receivers are typically employed when the treated region and spatial distribution of cavitation is relatively broad, such as during bulk ultrasound ablation [Mast et al., 2008b], cavitation threshold determination [Sassaroli and Hynynen, 2007, Gruber et al., 2014], and many ultrasound-enhanced drug delivery applications [Tang et al., 2002b, Datta et al., 2006, Qiu et al., 2010]. The development of techniques to calibrate focused receivers presents a greater challenge in comparison to that of unfocused receivers, yet the need for techniques to calibrate both types of receivers is significant.

Here, multiple substitution-type calibration techniques were investigated and developed for broadband calibration of various geometry, single-element transducers that are commonly employed as a PCD. First, these techniques were investigated numerically to determine configurations and parameters under which accurate broadband calibrations can be conducted from acoustic measurements alone, without the need for additional corrections for diffraction. These various techniques include a (1) bistatic scattering substitution technique developed by Collin and Coussios [Collin and Coussios, 2011] for calibrating focused receivers, as well as a (2) pitch-catch and a (3) pulse-echo technique developed here for calibrating focused and unfocused receivers. Second, the developed pulse-echo and pitch-catch techniques were employed to calibrate a focused receiver, and this calibration was validated with experimental measurements. Third, the receive system employed in Chapter 2
was fully characterized. This included calibration of an unfocused receiver employed as a PCD as well as characterization of the frequency response of the employed system electronics.

3.2 Methods

3.2.1 A bistatic scattering substitution technique for calibration of focused receivers: measurement overview and numerical analysis

Measurements to calibrate the receive sensitivity $M_R(f)$ of a spherically focused PCD using a bistatic scattering substitution method have previously been made using the measurement system shown in Figure 3.1(a) [Collin and Coussios, 2011]. In this method, a focused source is used to produce an acoustic pulse directed at a micron- or submicron-scaled spherical scatterer, and the scattered wave is measured by a confocally aligned PCD generating a voltage $X_R(f)$.

The reference pressure $\bar{P}_R(f)$ measurement is made by substituting the PCD with a calibrated needle hydrophone to make a static measurement in the exact orthogonal direction and at the same distance from the scatterer as the PCD. The receive sensitivity of the PCD is then determined by comparing the voltage of its measured scattering signal to the absolute pressure measured by the hydrophone following Equation 3.1. However, the accuracy of this calibration depends on the assumed equivalence between the hydrophone-measured scattered pressure and the scattered pressure spatially averaged by the PCD’s active surface.

Pressure signals received by a focused PCD and hydrophone were simulated to
represent measurements in the configuration shown in Figure 3.1(a) by calculating the frequency- and angle-dependent pressure scattered by a single sphere using an exact orthogonal function expansion [Faran Jr, 1951, Hickling, 1962] in MATLAB (R2014b, MathWorks, Natick, MA) using publicly available code [Anderson, 1999]. Scatterers were simulated as solid spheres composed of an isotropic, linearly elastic material and were assumed to be much smaller in diameter than the focal beamwidth of the source, so that the wave incident on the sphere could be approximated as a plane wave.

Scattered pressure was calculated as a function of the polar scattering angle $\theta_s$ and the dimensionless wavenumber $ka_s$, relative to the sphere radius $a_s$ and the wavenumber $k = 2\pi f/c$ where $c$ is the speed of sound in 25°C water. The focal length of the PCD, equal to its distance from the scatterer, was assumed great enough that the PCD surface $S_R$ resided in the far field of the simulated scattered wave. The frequency- and angle-dependent complex pressure $P_R(\theta_s, ka_s)$ incident over any point on the PCD surface was calculated using the far-field form function of the scattered wave [Hickling, 1962]. For a given PCD, the range of angle-dependent scattered pressures calculated across the PCD’s surface was geometrically determined by its $f$-number $N_R$, the ratio of the PCD focal length to its diameter, such that $\theta_s$ varied between $\cos^{-1}(\pm 1/(2N_R))$. The magnitude of the frequency-dependent pressure received by a PCD was calculated as the magnitude of the average complex scattered pressure incident over its surface as

$$|\bar{P}_R(ka_S)| = \frac{1}{S_R} \left| \int_{S_R} P_R(\theta_s, ka_S) \, dS \right|,$$  

(3.3)
where the polar scattering angle $\theta_s$ is any angle such that the far field pressure arrives at the surface of the receiver $S_R$. To represent the pressure received by a small-diameter hydrophone $|P_H(ka_s)|$, the magnitude of the complex pressure scattered at $\theta_s = 90^\circ$ was calculated over the same range of $ka_s$ values as

$$|P_H(ka_s)| = |P_R(90^\circ, ka_s)|. \tag{3.4}$$

This simulated point measurement was used as the reference pressure that would be used to approximate the magnitude of the PCD-measured pressure $P_R(f)$.

The scattered pressure measured by PCDs of $f$-number 0.5–8 and the corresponding point measurements representing that made by a small-diameter hydrophone were calculated for spherical scatterers composed of silica ($\text{sound speed } c_S = 5968 \text{ m/s, density } \rho_S = 2.20 \text{ g/cm}^3, \text{Poisson ratio } \nu_S = 0.17$) and polystyrene ($c_S = 2350 \text{ m/s, } \rho_S = 1.06 \text{ g/cm}^3, \nu_S = 0.34$), representing relatively rigid and compressible materials, respectively. Normalized sphere sizes of $ka_s$ up to 15 were investigated, corresponding approximately to frequencies in the megahertz range and scattering spheres of micron-scaled radii, consistent with previous calibration measurements using scattering techniques [Collin and Coussios, 2011, Sboros et al., 2005]. For both scatterer types, simulations of the spatially averaged PCD-measured and corresponding hydrophone-measured pressures were compared to test the equivalence of these measurements, and thus the achievable accuracy of calibrations using bistatic scattering substitution methods, as a function of frequency, PCD geometry, and scatterer type and size.
Figure 3.1: Schematic of transducer alignments for a (a) bistatic scattering technique with a scatterer $S$, (b) a pitch-catch, and (c) a pulse-echo substitution techniques for calibrating a focused, single-element passive cavitation detector (PCD).

3.2.2 A pitch-catch technique for calibration of focused receivers: measurement overview and numerical analysis

For a calibration of a focused transducer conducted using a pitch-catch technique, the open-circuit voltage $X_{R}(f)$ of the PCD is measured in a confocal pitch-catch configuration using a focused source to transmit a broadband pulse to the focused PCD. This configuration is schematically shown for a representative transducer pair of unequal geometry, with equal focal length and unequal diameter in Figure 3.1(b). The reference pressure $\bar{P}_{R}(f)$ is approximated from planar measurements made using a calibrated hydrophone at the same distance from the source as the PCD. This measurement is made by mapping the planar projection of the PCD and averaging the pressure magnitude measured over this region. The PCD sensitivity is then calculated using Equation 3.1 as the ratio of the open-circuit voltage...
generated by the PCD to the reference pressure measured by the hydrophone.

The measurements made in a pitch-catch calibration for a focused PCD were investigated numerically in MATLAB (2013a, Natick, MA USA) using an exact series solution to simulate the frequency-dependent complex pressure $P_T(f)$ field transmitted by a circular concave piston [Hasegawa et al., 1986]. First, the transmitted field was sampled over a concave surface corresponding to that of a confocally and coaxially aligned PCD. The amplitude of the average complex pressure incident over the simulated PCD surface $S_R$ was calculated to represent that received by a PCD as

$$\left| \bar{P}_R(f) \right| = \frac{1}{S_R} \left| \int_{S_R} P_T(f) \, dS \right|. \quad (3.5)$$

Second, the transverse plane at the same axial distance from the source was sampled over a circular diameter matching that of the PCD to represent potential hydrophone-measured pressures $P_H(f)$ for use as reference measurements. Simulated planar measurements were taken as the magnitude of the average complex pressure over a surface $S_P$, representing the planar projection of the investigated PCD, as

$$\left| \bar{P}_H(f) \right| = \frac{1}{S_P} \left| \int_{S_P} P_T(f) \, dS \right|. \quad (3.6)$$
or the average pressure magnitude

\[ |P_H(f)| = \frac{1}{S_P} \int_{S_P} |P_T(f)| dS. \quad (3.7) \]

For any calculation, a step size in any direction of approximately 1/2 of the smallest investigated wavelength was employed.

Simulated pressures spatially averaged across a concave and corresponding planar surface are shown as a function of normalized frequency \( ka_R \), relative to the wavenumber \( k \) in room temperature water and receiver radius \( a_R \), in Figure 3.2(a) for a confocal transmit-receive transducer pair with \( f \)-numbers 1.32 and 3.35, respectively. These dimensions matched those of the pair used later to implement the pitch-catch technique. The pressures averaged across the concave and planar surfaces were comparable at low \( ka_R \) values, but the amplitude of the average planar pressure is shown to diverge from the simulated PCD-measured pressure before approaching an average pressure of zero at higher \( ka_R \) values. However, the average pressure magnitude across the plane is shown to be approximately the same as the PCD-measured pressure for all investigated \( ka_R \) values, consistent with the assumption that the transmitted wavefront arrives at the PCD surface with approximately constant phase. Therefore, for the pitch-catch technique, the hydrophone was used to map the transverse plane of the source and the average pressure magnitude (Equation 3.7) over the plane was used as the reference measurement for calibration.

To illustrate the potential calibration accuracy using this reference pressure,
the average hydrophone measured magnitude was compared with the magnitude of the average pressure measured by a PCD for a variety of transmit-receive pairs of unequal geometry as a function of normalized frequency $ka_R$. Simulations were conducted employing a receiver of radius $a_R$ and $f$–number of 3.35 and a source with $f$–number 0.5–16.8 to represent potential transducer pairs for the pitch-catch technique. By varying the frequency of interest, the range of investigated $ka_R$ was varied over approximately 0–600.

### 3.2.3 A pulse-echo technique for calibration of focused and unfocused receivers: measurement overview and numerical analysis

For the calibration of a focused transducer conducted using a pulse-echo technique, the open-circuit voltage $X_R(f)$ of the PCD is measured in a pulse-echo configuration consisting of the PCD, used both as a source and receiver, and a rigid planar reflector at its focus. Alternatively, using the same alignment, an air-water interface could be employed instead of planar reflection plate. The pulse-echo configuration can be modeled by an equivalent confocal pitch-catch configuration of two identical transducers, as representatively shown in Fig. 3.1(c). Therefore, similar to the pitch-catch technique, the reference pressure $\bar{P}_R(f)$ is approximated from planar measurements made using a calibrated hydrophone. This measurement is made at a range distance equal to twice the focal distance of the PCD, that is also twice the separation distance between the PCD and the reflection plate. Hydrophone measurements are made over a the planar projection
Figure 3.2: Amplitude of simulated pressure spatially averaged over a concave PCD surface, $|\bar{P}_R(f)|$, pressure averaged over a corresponding hydrophone measurement plane, $|\bar{P}_H(f)|$, and pressure magnitude averaged over the same planar surface, $|P_H(f)|$. Pressures are plotted normalized to the nominal surface pressure amplitude of the source as a function of $ka_R$ for a (a) pitch-catch transducer pair with respective $f$-numbers of $N_T = 1.32$ and $N_R = 3.35$ and (b) a pulse-echo configuration with PCD $f$–number of $N_R = 3.35$.

Because a pulse-echo technique is analogous to a pitch-catch technique consisting of a transducer pair of equal geometry, the pulse-echo configuration was simulated as such by following the methods employed for pitch-catch technique for a transducer pair of equal geometry. The average complex pressure received by a PCD, as well as the average planar hydrophone-measured magnitude and complex pressures were calculated following Equations 3.5–3.7.
Simulated pressures spatially averaged across a concave and corresponding planar surface are shown as a function of normalized frequency $k a_R$, relative to the wavenumber $k$ in room temperature water and receiver radius $a_R$, in Figure 3.2(b) for an identical transducer pair with $f$–number 3.35, matching the geometry used later to implement the pulse-echo technique. Similar to the pitch-catch technique, the PCD-measured pressure corresponded across all investigated $k a_R$ values to the average pressure amplitude measured by the hydrophone, but not with the magnitude of the average hydrophone-measured pressure. Hence, for the pulse-echo technique, the hydrophone was used to map the transverse plane of the source and the average pressure magnitude (Equation 3.7) over the planar projection of the PCD’s image was used as the reference measurement for calibration.

To illustrate the potential calibration accuracy using this reference pressure for a pulse-echo technique, the average hydrophone-measured pressure magnitude was compared with the magnitude of the average complex pressure measured by a PCD for a variety of transducer pairs of equal geometry as a function of normalized frequency $k a_R$. Simulated transducer pairs consisted of equal geometry, 4 mm radius, with scaled focal lengths resulting in paired $f$–numbers of 0.5–12, representing potential PCD geometries for the pulse-echo technique. By varying the frequency of interest, the range of $k a_R$ was varied over approximately 0–700.

Following the same measurement methods as for a focused transducer, a pulse-echo technique can also be employed for calibrating unfocused receivers. However, the reflection plate may be placed orthogonally at any range distance within the transmit beam, including the far and near field. The open-circuit voltage $X_R(f)$
of the PCD is measured in a pulse-echo configuration consisting of the PCD, used both as a source and receiver, and a rigid planar reflector placed orthogonally to the transmitted pressure wave. The reference pressure $\bar{P}_R(f)$ is approximated from planar measurements made using a calibrated hydrophone by sampling a circular plane equal to the geometry of the PCD and at a distance equal to twice the separation of the PCD and the reflection plate. The average of the hydrophone-measured pressure magnitude is taken as the reference pressure measurement. Using the PCD-generated open-circuit voltage $X_R(f)$ and hydrophone-measured reference pressure $\bar{P}_R(f)$, the PCD sensitivity is calculated following Equation 3.1.

Because the measurement methods employed for calibrating focused transducers in a pulse-echo configuration is similar between focused and unfocused transducers, calibration measurements for an unfocused receiver were simulated using a pitch-catch configuration consisting of an equal geometry source and receiver. In practice, calibration measurements would be made by employing the same configuration used for a focused transducer calibration shown in Figure 3.1(c). However, because the focal region of a plane transducer is not fixed geometrically as for a spherically focused transducer, instead varying in the range distance due to frequency-dependent diffraction and interference, the location of the rigid reflection plate is not required to be precisely positioned at the focal distance of the transducer. An exact series expansion method [Mast and Yu, 2005] was employed in MATLAB to simulate the transmitted pressure field of a flat piston, single-element transducer representing a PCD. The reflected wave that would be received by the PCD in a pulse-echo configuration was simulated by calculating the transmitted pressure within a plane...
parallel to the source transducer surface at a range distance equal to twice that of
the range distance separating the source and reflection plate, over the PCD image
surface. The magnitude of the average complex pressure was taken over a circular
region of this plane with a radius equal to the radius $a_R$ of the simulated PCD.
The reference pressure measurement was simulated by sampling the same plane and
calculating the average pressure magnitude over the same circular region. Similar
to the case for focused transducers, the simulated hydrophone-measured average
pressure magnitude was used to represent reference pressure measurements. This
approximation, in comparison to the average complex pressure, is expected to be
less susceptible to inaccuracies induced by misalignment of the PCD or hydrophone.

The accuracy of this method, specifically using the average pressure magnitude
of the hydrophone-measured pressure to represent the average complex pressure that
would be received by a PCD, was investigated for various wavenumbers $k$ and PCD
radii $a_R$ simultaneously by varying the normalized frequency $ka_R$ of interest over a
range of 1.5–200. First, the agreement of simulated hydrophone- and PCD-measured
pressures were investigated under precise alignment conditions. These comparisons
were made for various separation distances between the PCD and measurement plane
of $z_0/a_R$ of 0.25–20 to identify if error would be induced due to the placement of
the reflection plate as a function of frequency and axial separation distance. Second,
the angle of alignment $\theta_M$ in a single axis of the receive plane was varied from 0–5°.
Simulated PCD- and hydrophone-measured pressures made under misalignment of
angle $\theta_M$ were compared to corresponding measured pressures made under perfect
alignment $\theta_0=0^\circ$ to identify the error induced by misalignment of the PCD with the
reflection plate or hydrophone. Third, the separation distance in the range direction between the PCD and hydrophone or reflection plate was varied over a range of $z_0/a_R$ of 0.4–12. Simulated PCD- and hydrophone-measured pressures made under a misaligned displacement of $z_M$, and normalized as $(z_0 + z_M)/a_R$, were compared to corresponding measured pressures made at the desired displacement of $z_0 = 65$ mm to identify error induced by misalignment in the range distance between the PCD and reflection plate or hydrophone.

The propagating pressure wave in the near field from a flat circular transducer can be approximated as a collimated plane wave of the same diameter as the transducer before expanding as a spherical wave in the far field. The transition point between the far and near field is commonly referred to as the Rayleigh length and is defined at an axial distance from the transducer as $z_R = S_R/\lambda = k a_R^2/2$ [Blackstock, 2000]. For a transverse plane measured in the field of a flat circular transducer at a given range position of $z$, a critical frequency, $k_0$, can be defined as the minimum frequency in which measurements in this plane are made within the near field by rearranging the Rayleigh length equation as $k_0 a_R = 2z/a_R$. For simulated measurements consisting of a 12 mm source and PCD, conducted at a range distance of $z = 65$ mm, the minimum frequency that can be measured within the near field is $k_0 a_R = 10.8$.

3.2.4 Characterization of a focused PCD using a pitch-catch and pulse-echo technique

Acoustic measurements were made in a tank of degassed, deionized water. The receive sensitivity of the same spherically focused, single-element transducer
(8.1 MHz center transmit frequency $f_0$, 4 mm radius $a_R$, 26.8 mm focal length; IMASONIC, Voray-sur-l’Oignon, France) was determined using pitch-catch and pulse-echo calibration methods over a frequency range of 1–20 MHz, corresponding to a $ka_R$ range relative to the PCD of approximately 17–340.

For the pitch-catch technique, a focused source (4.5 MHz $f_0$, 19 mm diameter, 25 mm focal length; UTX, Ithaca, NY USA) was confocally and coaxially aligned at a distance of 51.8 mm from the PCD and powered by a pulser-receiver (5052UAX50, Panametrics, Waltham, MA USA) to generate a broadband pulse. The time-dependent voltage generated by the PCD upon receiving the transmitted pulse was digitized by an oscilloscope (Waverunner 6050A, LeCroy, Chestnut Ridge, NY USA). This and all subsequent time-domain voltage acquisitions were made by temporally averaging 100 consecutive, 20 $\mu$s long waveforms sampled at 100 MHz by the oscilloscope. PCD voltage measurements were repeated independently 5 times, reconstructing the entire pitch-catch configuration for each measurement. The uncalibrated voltage $X_R(f)$ measurement in Equation 3.1 for the pitch-catch technique was calculated as the average magnitude of the fast Fourier transform (FFT) of repeated measurements.

For the pulse-echo technique, the PCD was aligned with a flat, 51 mm thick by 51 mm circular-diameter aluminum plate at its focus. The PCD and pulser-receiver were used in a pulse-echo mode and the reflected wave measured by the PCD was digitized by the oscilloscope. The frequency response of the pulser-receiver on receive was characterized by amplifying a white noise signal provided by a function generator (34401A, Agilent, Santa Clara, CA USA) and comparing
it in the frequency domain with the unamplified signal. Pulse-echo measurements were independently repeated 5 times, reconstructing the configuration for each measurement. After accounting for the frequency response of the pulser-receiver on receive and the reflection coefficient of the plate of approximately 0.7, the absolute value of the FFT of each measurement was calculated and the average of the multiple trials was used as the uncalibrated voltage $X_R(f)$ measurement in Equation 3.1 for the pulse-echo technique.

All reference measurements were made using a manufacturer-calibrated hydrophone (0.5 mm diameter; 1239, Precision Acoustics, Dorchester, Dorset, UK). The same sources used for PCD-voltage measurements, excited by the pulser-receiver in the same manner, were used for the reference measurements for each calibration technique. For the pitch-catch technique, the PCD was substituted with the hydrophone and the acoustic field was mapped in the transverse plane at an axial distance of 51.8 mm from the source, equal to the sum of the focal lengths of the focused transducer pair. For the pulse-echo technique, the reflection plate was removed and the transverse plane of the PCD’s transmit field was mapped at an axial distance of 53.6 mm, equal to twice its focal length. Both planes were sampled over an 8 mm circular diameter, matching the planar diameter of the PCD, with a 0.2 mm spatial resolution in either azimuthal direction by scanning the hydrophone across the plane using a 3-D stepper motor system (Velmex, Bloomfield, NY, USA). Time-dependent voltages were digitized at each planar position by the oscilloscope. The spatial average of the absolute value of the FFT of the signal measured at each planar position was taken to determine
the average open-circuit voltage $|V_H(f)|$ amplitude measured by the hydrophone. The average pressure amplitude measured by the hydrophone was calculated as $|P_H(f)| = |V_H(f)|/M_H(f)$, where $M_H(f)$ is the hydrophone’s receive sensitivity, provided by the manufacturer over the frequency range 1–20 MHz.

The average hydrophone-measured pressure magnitude $|P_H(f)|$ was used to approximate the magnitude of the average pressure received by the PCD $P_R(f)$. Using this value with the open-circuit voltage $X_R(f)$ generated by the PCD, the frequency-dependent absolute receive sensitivity of the PCD was determined for both calibration methods following Equation 3.1. Frequency-dependent uncertainty, associated in part with alignment variations, was estimated from the coefficient of variation of repeated PCD voltage measurements. This uncertainty was propagated with the manufacturer-specified uncertainty for the hydrophone sensitivity to calculate the total, frequency-dependent uncertainty of the PCD’s receive sensitivity. Uncertainties in measured pressures were estimated as the frequency-dependent pressures multiplied by the frequency-dependent receive sensitivity uncertainties of the hydrophone and PCD respectively.

Validation measurements were conducted using a 2 mm diameter barrel-shaped transducer (1.4 MHz $f_0$; Sonometrics, London, Ontario, CA) as a source, aligned coaxially with the hydrophone or PCD at a distance of 26.8 mm, the focal distance of the PCD, to approximate a point radiator. The source was excited by the pulser-receiver using four energy settings to generate broadband pulses of different amplitude. Using the PCD as a receiver, the voltage generated upon receiving each pulse was digitized by the oscilloscope and the open-circuit voltage $X_R(f)$
of the PCD was calculated as the absolute value of the FFT of the acquired signal. Using the receive sensitivity $M_R(f)$ of the PCD determined from the pitch-catch and pulse-echo methods, the frequency-dependent pressure amplitude of each pulse measured by the PCD was calculated following Equation 3.1. The absolute, frequency-dependent pressure of the each pulse was determined using the hydrophone as in the calibration methods by scanning the transverse plane at the same distance, taking the average of the absolute value of the FFT of each signal, and taking the ratio of this voltage to the hydrophone sensitivity. Measured pressures were compared as a function of frequency up to approximately 10 MHz, the approximate maximum useful radiating frequency of the barrel-shaped source.

### 3.2.5 Sonophoresis: Characterization of receive system sensitivity and frequency response

The absolute receive sensitivity of an unfocused, flat piston transducer (C302, S/N 1035098, $f_0 = 1$ MHz; Panametrics, Waltham, MA USA) was calibrated using a pulse-echo calibration technique. This transducer was identical in geometry ($a_R = 12.7$ mm), model (C302, S/N 542754), and frequency bandwidth to the transducer ($f_0 = 1$ MHz, Panametrics) employed as a PCD in Chapter 2 during sonophoresis.

Acoustic measurements were made by aligning the PCD with a flat, 51 mm thick by 51 mm circular-diameter aluminum plate at a range distance of 32.5 mm. The PCD and pulser-receiver were used in a pulse-echo mode and the reflected wave measured by the PCD was digitized by the oscilloscope. Pulse-echo measurements were independently repeated 10 times, reconstructing the
configuration for each measurement. The frequency response of the pulser-receiver on receive was characterized by amplifying a white noise signal provided by a function generator (34401A, Agilent, Santa Clara, CA USA) and comparing it in the frequency domain with the unamplified signal. After accounting for the frequency response of the pulser-receiver on receive and the reflection coefficient of the plate of approximately 0.7, the absolute value of the FFT of each measurement was calculated and the average of the multiple trials was used as the uncalibrated voltage $X_R(f)$ measurement.

Reference pressure measurements were made using two hydrophones; one hydrophone was employed to measure frequencies less than 1 MHz (TC4038, Reson, Slangerup, Denmark) and another to measure frequencies greater than 1 MHz (0.5 mm diameter; 1239, Precision Acoustics, Dorchester, Dorset, UK). For either hydrophone, a circular plane of 12.7 mm radius was mapped at a range distance of 65 mm from the PCD. The PCD was driven to generate the same waveform as that generated for the reflection measurements. The average magnitude of the hydrophone-measured pressure $|P_H(f)|$ over the circular plane was calculated and used to approximate the magnitude of the pressure $|P_R(f)|$ received by the PCD. The frequency-dependent receive sensitivity was then calculated following Equation 3.1 as the ratio of the hydrophone reference pressure $|P_H(f)|$ to the PCD-generated voltage $X_R(f)$.

Frequency-dependent uncertainty was estimated from the coefficient of variation of repeated PCD voltage measurements. Repeated measurements were made by reassembling and aligning the reflection configuration 10 times and measuring
the reflected wave for each replication. The uncertainty in these measurements was propagated with the manufacturer-specified uncertainty for the associated hydrophone sensitivities to calculate the total, frequency-dependent uncertainty of the receive sensitivity for the PCD.

In addition to the characterization of the PCD receive sensitivity, the frequency response of the employed electronics is required in order to make absolute pressure measurements. First, the frequency response $G_R(f)$ of the electronic components employed for PCD measurements during sonophoresis in Chapter 2 (Figure 2.1) was measured. A white noise signal generated by a function generator (Agilent, Santa Clara, CA USA) was passed through an isolation transformer (M0203, TTE Inc., Los Angeles, CA USA) inline with a preamplifier (SRS560, Stanford Research Systems, Sunnyvale, CA USA). To replicate the settings applied during intermediate-frequency sonophoresis (IFS, $f_0 = 0.41$ MHz), the preamplifier was configured to apply a 10–300 kHz band-pass filter and amplify filtered signals by a factor of 100. To replicate the settings applied during high-frequency sonophoresis (HFS, $f_0 = 2.0$ MHz), the preamplifier was configured to apply a 10–1000 kHz band-pass filter and amplify filtered signals by a factor of 500. The frequency response of these components was characterized by comparing the filtered and amplified output signal to the original input signal.

Second, the absolute receive sensitivity $M_R(f)$ of the transducer employed as a PCD was determined. This was conducted using the calibration of a transducer (C302, S/N 1035098; Panametrics, Waltham, MA USA) with identical geometry ($a_R = 12.7$ mm), model (C302), center frequency ($f_0 = 1$ MHz), and bandwidth to
the transducer (S/N 542754, Panametrics) employed for sonophoresis in Chapter 2. To determine the receive sensitivity of the latter (C302, S/N 1035098), the energy in pulse-echo waveforms generated by each transducer and provided by the manufacturer were compared. Due to the nearly identical frequency response of these transducers, the sensitivity of the former was linearly scaled by the difference in the signal energy within pulse-echo measurements in order to determine the sensitivity of the latter. The resultant difference between pulse-echo measured energies was approximately a factor of 2, hence the sensitivity of the former was scaled by the square root of 2 to determine that of the latter. Due to the similarities between transducers, the frequency-dependent uncertainty calculated for the former was assumed to be equivalent to that of the latter and was propagated directly.

3.3 Results

3.3.1 Accuracy of a bistatic scattering substitution technique for focused receivers

Figure 3.3 illustrates the far-field scattered waves from silica and polystyrene spheres as a function of $ka_s$ and scattering angle $\theta_s$. The scattered field amplitude is shown in Figures 3.3(a) and 3.3(c) as decibel-scaled values relative to the maximum scattered pressure, while the scattered field phase is shown in Figs. 3.3(b) and 3.3(d) for a silica and polystyrene scatterer, respectively. For both scatterer types, the directivity of scattered sound becomes more complex with increasing $ka_s$ with greater angle-dependent variations in phase and amplitude. Thus, except over a limited range of relatively low $ka_s$ values, the scattered pressure measured by a
Figure 3.3: The amplitude and phase of the far-field scattered pressure by a silica (a,b) and polystyrene (c,d) sphere, respectively, are shown as a function of $ka_s$ for scattering angles $\theta_s$ of 0-180°. Pressure amplitude values are shown as dB-scaled values relative to the maximum calculated pressure. The frequency-dependent wave measured across the surface of a PCD receiver with $f$–number $N_R$ corresponds to scattering angles centered at $\theta = 90^\circ$, the location of hydrophone measurements, and varying between $\cos^{-1}(\pm1/(2N_R))$. 
small diameter hydrophone at $\theta_s = 90^\circ$ will substantially differ from that measured by low $f$–number PCDs, represented by spatial averaging over a broader range of scattering angles about $\theta_s = 90^\circ$. For PCDs of higher $f$–number, corresponding to a narrower range of scattering angles about $\theta_s = 90^\circ$, PCD- and hydrophone-measured pressures will correspond accurately for larger values of $ka_s$.

Shown in Figures 3.4(a) and (b) are dB-scaled amplitude ratios of the simulated PCD-measured scattered pressure to the simulated hydrophone-measured pressure for silica and polystyrene spheres, respectively, as a function of $kas$ and PCD $f$-number $N_R$. Dashed and solid lines in each figure show contours of amplitude ratios $\pm 1$ dB and $\pm 3$ dB as representative accuracy limits. Within these representative accuracy limits for both scatterer types, the viable range of $kas$ for accurate calibration increases with PCD $f$-number. For any given PCD $f$-number, the silica scatterer enables higher calibration accuracy over a greater range of sphere sizes.

Shown in Figures 3.5(a) and (b) are the minimum $kas$ values resulting in amplitude ratios greater than $\pm 1$ dB and $\pm 3$ dB for a silica and polystyrene scatterer, respectively. For comparison, dotted lines in each plot show values associated with sphere diameters equal to 1, $1/2$ and $1/4$ of the PCD’s $-6$ dB focal beamwidth for different $kas$ values as a function of the PCD $f$-number. The $-6$ dB beamwidth at the focus of the PCD was calculated under the Fresnel approximation as

$$BW_{-6 \text{ dB}} = 2.8\pi \frac{N_R}{k}.$$  \hspace{1cm} (3.8)
Figure 3.4: (a) Decibel-scaled ratio of simulated PCD-measured to hydrophone-measured pressure for a silica glass sphere as a function of normalized sphere size $ka_S$ and PCD $f$-number $N_R$. Dashed and solid lines indicate $ka_S$ values resulting in ratios $\pm 1$ or $\pm 3$ dB, respectively. (b) Corresponding pressure ratios and contours for a polystyrene sphere.

For both scatterers, the maximum sphere size for PCD calibration within a given accuracy limit can be approximately expressed as a fraction of the $-6$ dB PCD focal beamwidth. For a silica scatterer (Figure 3.5(a)), in order to maintain $\pm 1$ dB agreement between PCD and hydrophone measurements, the sphere diameter must be less than about $1/2$ PCD beamwidth over the frequency range of interest. To maintain $\pm 3$ dB agreement, the silica sphere diameter must be less than about one PCD beamwidth. To maintain $\pm 1$ and $\pm 3$ dB agreement between measurements for the polystyrene scatterer (Figure 3.5(b)), the sphere diameter must be less than approximately $1/4$ and $1/2$ PCD focal beamwidth, respectively.
Figure 3.5: (a) Minimum $ka_S$ values exceeding pressure ratios of $\pm 1$ dB (solid line) and $\pm 3$ dB (dashed line) for a silica sphere, as a function of $N_R$. Dotted lines indicate $ka_S$ values corresponding to scatterer diameters 1, 1/2, and 1/4 the corresponding $-6$ dB PCD focal beamwidth. (d) Corresponding plots for a polystyrene sphere.

### 3.3.2 Accuracy of a pitch-catch and pulse-echo technique for focused receivers

The absolute value of the dB-scaled ratio of average hydrophone-measured pressure magnitude to the magnitude of the average complex pressure measured by a PCD is shown for a variety of transmit-receive pairs of unequal and equal geometry in Figure 3.6(a) and (b), respectively. These results represent the accuracy of measurements made for a pitch-catch and pulse-echo technique, respectively.

For transducer pairs consisting of a source of greater $f$–number than the receiver, the hydrophone-measured pressure significantly exceeded that measured by the PCD and this difference increased with increasing source $f$–numbers,
Figure 3.6: The dB-scaled ratio of simulated PCD-received and corresponding hydrophone-measured pressures as a function of frequency for (a) transducer pairs of unequal geometry employed in a pitch-catch calibration configuration and (b) transducer pairs of equal ($N_T = N_R$) geometry in a pulse-echo calibration configuration. The solid and dashed lines contour regions in (a) that exceed ±3 and ±1 dB difference between measurements.

especially at higher $ka_R$ values as shown in Figure 3.6(a). However, for transducer pairs consisting of a source with equivalent or smaller $f$-number than the PCD, the difference between measurements was no more than 0.45 dB over the range of investigated $ka_R$ values. This indicates that a broadband calibration can be conducted by approximating the PCD-received pressure from planar hydrophone measurements, with small inherent error between the measurements, when the source is of equivalent or small $f$-number than the PCD to be calibrated.

For equal geometry transducer pairs with an $f$-number less than 1, the PCD-measured pressure was overestimated by the hydrophone-measured pressure by no
more than 1.3 dB over the range of investigated $ka_R$ values as shown in Figure 3.6(b). However, the difference between measurements was less for transducer pairs with an $f$-number greater than 1, exceeding a difference of no more than 0.7 dB over the same $ka_R$ range. This indicates that a broadband calibration can be conducted by approximating the PCD-received pressure from planar hydrophone measurements, with minimal inherent error between the measurements of less than 1.3 dB, for any focused transducer. This additionally indicates that this method can be employed for the calibration of unfocused, flat transducers since the broadband accuracy increases as the PCD surface is flattened with increasing $f$-number.

### 3.3.3 Accuracy of a pulse-echo technique for unfocused receivers

Shown in Figure 3.7(a) is the dB-scaled agreement between the average pressure magnitude and the magnitude of the average complex pressures representing measurements made by a hydrophone and PCD, respectively, in a pulse-echo configuration for calibrating the receive sensitivity of a flat piston transducer. This agreement is shown for various path-length distances separating the PCD and the hydrophone-measured plane $z_0/a_R$ or reflection plate $z_0/2a_R$ as a function of normalized frequency $ka_R$. Over this parameter range, the disagreement between measurements was limited to no more than 0.4 dB with the hydrophone-measured pressure exceeding that which would be measured by the PCD. This indicates that the value resulting from taking the average pressure magnitude, in comparison to
Figure 3.7: (a) The decibel-scaled agreement between hydrophone-measured average pressure magnitudes ($P_H$) and PCD-measured average complex pressures ($P_R$) for different distances ($z_0/a_R$) separating the PCD and measurement plane as a function of normalized frequency ($ka_R$). The blue line indicates the minimum normalized frequency, $k_0a_R$, measured within the near field for a given separation distance $z_0/a_R$. (b) Representative $P_R$ and $P_H$ values as a function of normalized frequency measured for a 12 mm circular radius $a_R$ PCD at range position of $z_0 = 65$ mm.

taking the average complex pressure, in the hydrophone-measured plane closely approximates the magnitude of the average complex pressure measured by the PCD. Additionally shown as a solid blue line is the minimum normalized frequency, $k_0a_R$, that can be measured within the near field for any given separation distance, $z_0/a_R$, between the source and measurement plane. That is, measurements made for a $ka_R$ value greater than that indicated by the line were conducted within the near field of the source. Notably, this figure illustrates that for measurements made with precise alignment, calibration measurements can be made for a plane circular transducer
with error less than 0.4 dB when conducted within the near or far field. For reference, measurements made for a transducer of circular radius of $a_R = 12$ mm at a range distance of $z = 65$ mm correspond to measurements in Figure 3.7(a) at $z/a_R = 5.4$.

Figure 3.7(b) shows simulated pressure measurements specifically for the case of a PCD with a circular radius of $a_R = 12$ mm and for a hydrophone measured-plane at $z_0 = 65$ mm ($z_0/a_R = 5$) as a function of normalized frequency $k a_R$, corresponding to a frequencies range up to 4 MHz for this PCD. This specific configuration was that used for calibration measurements for the unfocused transducer employed during sonophoresis in Chapter 2. For this specific case, the difference in pressure measurements was limited to a maximum of 0.29 dB disagreement, with the hydrophone-measured value exceeding that which would be measured by a PCD, over the investigated range of frequencies. Although minimal, this disagreement is inherent to this calibration method and is due to the approximation used to represent the PCD-measured pressure. However, this comparison only displays results measured under precise alignment and the error induced over the complex pressure field grows rapidly with misalignment.

Shown in Figure 3.8(a) is the dB-scaled ratio of the amplitude of the average complex pressure measured at a desired range location of $z_0$ relative to that measured at a misaligned range position of $z_M$, normalized by the transducer radius $a_R$, as function of normalized frequency $k a_R$. Regions of ±1 and ±3 dB disagreement are indicated by red dotted and dashed lines, respectively. These comparisons represent error that may be induced by not correctly aligning the source with a PCD or reflection plate in the range direction for a pitch-catch and pulse-echo measurement,
respectively. Except for at low frequencies, the disagreement between the desired measurement and misaligned measurements for this configuration is shown to be relatively low even for large misalignment in range displacement. Further, although calibration measurements for plane circular transducers can be made accurately in the near or far field (Figure 3.7(a)), the results shown in Figure 3.8(a) suggest that measurements may be less susceptible to error induced by axial misalignment when conducted in the near field for all frequencies.

The solid blue line in Figure 3.8(a) corresponds to the critical normalized frequency value, $k_0a_R$, at a given range separation distance associated with simulated measurements where the misaligned plane at $z_M$ was measured at a greater distance from the source than the reference plane at $z_0$. For these measurements, the reference and misaligned plane were both measured in the near field only for range separation distances that correspond with $ka_R$ values greater than the critical value $k_0a_R$ indicated by the line. For a measurement plane at range distance $z_0 = 65$ mm in the field of a transducer with a radius $a_R = 12$ mm, the minimum frequency at which the reference plane can be measured within the near field is $k_0a_R = 10.8$. The blue line is shown as a constant $ka_R$ value of 10.8, the associated $k_0a_R$ value for the simulated configuration, for simulated measurements where the misaligned plane at $z_M$ was measured at a distance closer to the source than the reference plane at $z_0$. For these measurements, the reference and misaligned planes were both measured in the near field only at range separation distances that correspond with a $ka_R$ value greater than 10.8, the critical frequency for this configuration.

The error due to axial misalignment for measurements made in the near field
Figure 3.8: Error induced on a pulse-echo or pitch-catch calibration measurements by misaligning the PCD in (a) the range distance by a distance $z_M$ or (b) axial angle $\theta_M$. All values are shown as the decibel-scaled ratio of the average complex pressure measured by an aligned PCD ($P_{R}(z_0 = 65 \text{ mm}, \theta_0 = 0^\circ)$ to the misaligned measurement ($P_{R}(z_M, \theta_M)$) as a function of normalized frequency. In Figure (a), red dotted and dashed lines indicate contoured regions of ±1 and ±3 dB error, respectively. The blue line indicates the minimum normalized frequency, $k_0a_R$, for measurements made within the near field for a given separation distance. In Figure (b), dotted, dashed, and solid lines indicate contoured regions of ±1, ±3, and ±6 dB error, respectively.

were relatively low and typically below ±1 dB, even for large axial misalignment distances, as shown in 3.8(a). However, in comparison, when one or both of the measurements were made within the far field, at $ka_R$ values less than that indicated by the solid blue line, measurement error due to axial misalignment increased more rapidly with misalignment. Specifically, ±1 dB error due to axial misalignment approximately corresponds to when either measurement was made within the far
field, as indicated for measurements made at any range separation distance for frequencies, \( ka_R \), less than the critical value indicated by the line. Hence, calibration error due to misalignment in the range direction can be reduced by conducting measurements within the near field of the lowest frequency of interest.

In Figure 3.8(b), the dB-scaled difference between PCD-measured average complex pressures are shown for measurements made with a perfect alignment (i.e., \( \theta_0 = 0^\circ \)) relative to those made with the PCD misaligned in one axis by an angle \( \theta_M \) as function of normalized frequency \( ka_R \). Dotted, dashed, and solid lines contour regions that the agreement exceeded 1, 3, and 6 dB respectively. In comparison to the disagreement rendered by misalignment in range position \( z \), the average complex pressure value is substantially more sensitive to angular misalignment. For example, a misalignment by just \( 1^\circ \) for a \( ka_R \) of 200 results in greater than 10 dB of error. Measurements made at \( ka_R \) values less than 10.8 for this configuration were made within the far field. However, unlike the error due to axial misalignment, error due to the misalignment of the axial angle is not as sensitive to the position within the field that measurements are made. Instead, this error is primarily due to variations in the phase cancellation in the average complex pressure over the measurement plane that are induced by the tilt angle of the PCD, requiring precise angular alignment for accurate calibration measurements. In addition, Figure 3.8(b) illustrates that for transducers of small radius, \( a_R \), and for lower frequencies, \( k \), calibration measurements are less sensitive to angular alignment, independent of the location within the field that measurements are made.

Shown in Figures 3.9(a) and 3.9(b) are examples of the hydrophone-measured
Figure 3.9: Error induced on a pulse-echo calibration by the hydrophone measured plane being misaligned in the (a) range distance by $z_M$ or (b) axial angle $\theta_M$. All values are shown as the decibel-scaled ratio of the average pressure magnitude measured by an aligned hydrophone ($P_H(z_0, \theta_0)$) to the misaligned measurement ($P_H(z_M, \theta_M)$) as a function of normalized frequency. In (a), dotted and dashed lines indicate contoured regions of ±1 and ±3 dB error, respectively. The blue line indicates the minimum normalized frequency, $k_0 a_R$, for measurements made within the near field for a given separation distance.

average pressure magnitudes measured in misalignment in range position $z_M$ and axis angle $\theta_M$ relative to PCD-measured average complex pressures measured in perfect alignment in range position $z_0$ and axis angle $\theta_0$, respectively. Dotted and dashed lines shown in Figure 3.9(a) contour regions of ±1 and ±3 dB. Additionally the blue line in Figure 3.9(a) corresponds to the the critical frequency $k_0 a_R$ associated with a given range displacement. This line corresponds to that also shown in Figure 3.8(a) to indicate measurement pairs conducted within the near field for any $ka_R$ value equal to or greater than those marked by the solid line. Notably, the error
induced by misalignment in the range position of the hydrophone-measured plane increases with misalignment most notably for when the reference, misaligned, or both measurements are conducted within the far field. Further, as indicated for the PCD measurements in Figure 3.8(a), error due to misalignment in the range position can be minimized by conducting measurements at a maximum axial separation distance equal to the Rayleigh length associated with the lowest frequency of interest.

Shown in Figure 3.9(b) is the dB-scaled difference between hydrophone-measured pressures made with a perfect alignment (ie, $\theta_0 = 0^\circ$) relative to those made with the hydrophone plane misaligned in one axis by an angle $\theta_M$ as function of normalized frequency $ka_R$. In comparison to the PCD-measured pressures made under misalignment in the axial angle (Figure 3.8(b), the error induced by measuring the hydrophone plane under misalignment is substantially less. Over the investigated parameter space, axial angle misalignment up to a $\theta_M = 5^\circ$ and frequency of $ka_R = 200$, the maximum error relative to the complex pressure that would be measured by a perfectly aligned PCD was 0.315 dB. These results indicate that average pressure magnitude measured by a hydrophone provides an accurate approximation of the PCD-measured pressure, and by making this approximation the measurements are less prone to misalignment error. The minimization of alignment error for the hydrophone measurements in comparison to those made by a PCD is due to the absence of phase cancellation for hydrophone measured pressures, and due to the amplitude of the pressure field varying slowly. The absence of phase cancellation, due to measuring the average pressure amplitude instead of the complex value, leads to a reduction of error sensitivity to the angle of alignment of the hydrophone.
plane, even for axial misalignment angles of up to 5°. Hence, even for plane circular transducers for which the surface can more easily be traced by a hydrophone, the average pressure magnitude is used as the reference pressure measurement instead of the average complex value in order to reduce alignment errors.

### 3.3.4 Receive calibration of a focused receiver using a pulse-echo and pitch-catch technique

Following the methods of Section 3.2.4, the frequency-dependent voltage amplitudes generated by the focused PCD in the pitch-catch and pulse-echo configuration and the corresponding average voltage amplitudes of the planar hydrophone measurements were measured and are shown in Figures 3.10(a) and 3.10(b) for the pulse-echo and pitch-catch techniques, respectively. Voltage measurements using the pulse-echo technique provided a calibration bandwidth of approximately 20 MHz, limited by the calibration bandwidth of the hydrophone. Although the SNR of measurements using the pitch-catch technique approached the noise floor within a narrower bandwidth, calibration was still feasible over a bandwidth of approximately 16 MHz.

The absolute receive sensitivity of a focused PCD determined using a pitch-catch and pulse-echo technique is shown as a function of frequency in Figure 3.11 over the usable bandwidth for each method. Error bars shown indicate the calculated frequency-dependent uncertainty of the PCD’s receive sensitivity for each calibration method. The frequency-averaged uncertainty in the PCD receive sensitivity for the pulse-echo and pitch-catch techniques was 23.4% and 23.2%,
respectively. The contribution of uncertainty from the hydrophone measurements alone, based on values provided by the manufacturer, was on average 19.7% across its calibrated bandwidth. For comparison, the uncertainty contributed from repeated PCD voltage measurements over the bandwidth of each calibration was 12.6% and 12.2% for the pulse-echo and pitch-catch techniques, respectively.

Frequency-dependent pressures measured from the 2-mm validation source by the PCD, calculated using the receive sensitivities determined using the pulse-echo and pitch-catch techniques, are compared with corresponding hydrophone-measured pressures in Figure 3.12 for a representative trial using the highest of the four voltage amplitudes employed to drive the validation source. Relatively good overall correspondence between the hydrophone- and PCD-measured pressures is seen, but considerable differences are evident near higher-frequency nulls, e.g. at approximately 8 MHz. Mapping the transmit field within these anomalous

Figure 3.10: Frequency-dependent voltages measured by a PCD and hydrophone for (a) pulse-echo and (b) pitch-catch technique. These measurements illustrate the usable bandwidth for calibration of the PCD.
frequency ranges revealed asymmetric phase patterns, which caused significant
destructive interference across the PCD surface. Since these aberrations were
inherent to the transmit field of the source and not to the calibration or
validation methods, the pressures associated with these null-producing frequencies
were neglected for comparison purposes. Instead, for the four different transmit
conditions, pressure values were sampled at frequencies associated with symmetric
phase maps which more closely resembled those produced by a point radiator
at the focus. The frequency bins sampled for further analysis are indicated by
error bars in Figure 3.12. Pressure values measured at these frequencies by the
PCD using the pulse-echo and pitch-catch techniques are directly compared with
corresponding hydrophone-measured pressures in Figure 3.13. All error bars in
Figure 3.13 represent the dB-scaled, frequency-dependent calculated uncertainty
Figure 3.12: Representative frequency-dependent pressures measured by a hydrophone and PCD, with error bars representing the uncertainty associated with each receive sensitivity. Displayed PCD-measured values were determined using sensitivities determined using a pitch-catch and pulse-echo method.

of pressure measured by each respective receiver. The gray box represents the dB-scaled average uncertainty of the hydrophone plotted above and below the one-to-one line for comparison with the PCD-measured pressures.

3.3.5 Receive system characterization and calibration of an unfocused receiver by a pulse-echo technique

The solid line in Figure 3.14(a) shows the normalized dB-scaled voltage measured by the PCD in pulse-echo. The gray shaded region represents the frequency-dependent standard deviation calculated over 10 independent measurements. This signal was used in part to determine the usable bandwidth of the transducer signal for calibration. For this PCD, the usable bandwidth was found to be approximately 0.07–2.5 MHz. Outside of this range, the signal approaches the noise-floor as well.
as the deviation in the voltage measurements begins to exceed reasonable values. Over the chosen bandwidth, the uncertainty in repeated voltage measurements was on average 12.75%.

Shown in Figure 3.14(b) is the measured dB-scaled uncertainty (solid line), calculated as the maximum measured voltage relative to the minimum over the 10 repeated measurements, as a function of frequency. Also shown is the uncertainty estimated in simulation for the same PCD and configuration due to a 1° axial misalignment (dashed line). Due to the agreement between the simulated and measured uncertainties over the bandwidth of interest, the uncertainty associated with repeated measurements can be primarily attributed to misalignment of the
transducer of up to 1° axial misalignment.

The absolute receive sensitivity of two flat piston-type transducers is shown in Figure 3.15. These receive sensitivities correspond to the transducer (S/N 1035098) that was calibrated using the pulse-echo technique and that which was estimated for the transducer (S/N 542754) employed as a PCD for sonophoresis. Error bars for each indicate the total propagated frequency-dependent uncertainty associated with repeated voltage measurements and the uncertainty in the sensitivity of the manufacturer calibrated hydrophone. The frequency-averaged uncertainty in the receive sensitivities was 29.1% over the calibration bandwidth of approximately 0.07–2.5 MHz for either transducer. The contribution of uncertainty from the hydrophone measurements alone, based on values provided by the manufacturers
Figure 3.15: Frequency-dependent, absolute receive sensitivity of two unfocused transducers (C302, S/N 1035098 and S/N 542754) calibrated using a pulse-echo technique. Error bars shown at representative frequencies indicate the total uncertainty of the calibration.

of each hydrophone, was on average 24.3% across the calibration bandwidth. For comparison, the uncertainty contributed from repeated PCD voltage measurements over the same bandwidth was 12.75%. The total calibration uncertainty at 0.205 and 1 MHz, the subharmonic associated with intermediate- (IFS) and high-frequency sonophoresis (HFS) trials conducted in Chapter 2, was 39.68% and 19.42%, respectively. Additionally, in comparison to the focused PCD calibrated using the same technique (Figure 3.11), the receive sensitivity of the unfocused transducer is approximately 20 times less sensitive than the focused PCD.

Shown in Figure 3.16(a) is the frequency-dependent decibel-scaled gain $G_{sys}$ provided by the electronics, accounting for filtering and amplification of PCD-generated voltages, used for sonophoresis in Chapter 2. The gain values are shown for the electronic components and settings used for IFS and HFS trials individually.
Figure 3.16: (a) The frequency response $G_{sys}$ of the system electronics as a function of frequency used for IFS and HFS. (b) The total system response $M_{sys}$, the product of the response of the system electronics $G_{sys}$ and PCD receive sensitivity $M_R$, as a function of frequency for IFS and HFS configurations. The total system response $M_{sys}$ is the transfer function that can be used to convert the receive system-measured voltages to absolute pressure values.

as a function of frequency over the range associated with the bandwidth of the calibration of the employed PCD. In Figure 3.16(b) is the total sensitivity $M_{sys}$ of the receive system employed for IFS and HFS trials, shown as dB-scaled values as a function of frequency. This sensitivity is calculated as the product of the individual contributions from the receive system electronics $G_{sys}(f)$ (Figure 3.16(a)) and the receive sensitivity $M_R(f)$ of the PCD (Figure 3.15).

3.4 Discussion and conclusions

3.4.1 A bistatic scattering substitution calibration technique
In this study, a bistatic scattering substitution method for calibrating the absolute, broadband receive sensitivity of a PCD was numerically investigated. The accuracy of this method was shown to be sensitive to the calibration frequency, PCD dimensions, and scatterer size and material. Best calibration accuracy is achieved when the scattered wave has approximately constant phase and amplitude at the PCD surface, corresponding to lower ultrasound frequencies, higher PCD $f$-numbers, and smaller scatterers. In particular, for a given scatterer type and frequency, if the sphere diameter is sufficiently smaller than the focal beamwidth of the PCD, the received pressure will match that measured by the hydrophone with relatively high accuracy.

Accuracy limitations were shown to be induced by non-uniform scattering from a silica and polystyrene sphere, resulting in discrepancies between the pressure averaged across the PCD surface and the pressure received by the corresponding small hydrophone. This discrepancy was shown to increase in magnitude as the PCD $f$-number decreases and as the frequency or scatterer diameter increases. Within limits of acceptable accuracy, represented in Figure 3 as $\pm 1$ and $\pm 3$ dB ratios between PCD- and hydrophone-measured pressures, broadband calibration measurements could be conducted for any given PCD geometry with careful consideration of the scatterer material and size. In general, accurate calibrations are feasible over wider frequency ranges for larger PCD $f$-numbers and for more rigid scattering materials.

For the calibration reported by Collin and Coussios [Collin and Coussios, 2011], a PCD with a focal beamwidth of approximately 400 $\mu$m at 20 MHz, the highest
investigated frequency, was calibrated using a 100 µm diameter silica sphere. The sphere diameter was thus approximately 1/4 of the focal beamwidth at the highest frequency calibrated. The simulation results shown here suggest that the accuracy of this calibration over the investigated bandwidth was relatively high, with agreement between PCD- and hydrophone-measured pressures likely less than ±1 dB.

In cases causing large discrepancies between PCD and hydrophone pressure measurements, calibration accuracy may be improved by numerical correction of the calibration methods if the properties of the scatterer are known. A correction could be implemented by computing the ratio of the simulated scattered pressure across the PCD to that of the hydrophone, as shown here in Figure 3(a) and 3(b), over the $k_{as}$ range of interest for the specific PCD and scatterer. Using this calculated ratio, measured values could be numerically compensated to improve calibration accuracy. Additionally, the reference scattered pressure could be simulated instead of measured, with scaling based on hydrophone measurements of the incident wavefield at the sphere location, similar to a method used to calibrate phased array transducers. [Sboros et al., 2005]

### 3.4.2 Pitch-catch and pulse-echo calibration techniques for focused receivers

Numerical and experimental results shown in Section 3.3.4 demonstrate that the wideband, absolute receive sensitivity of the spherically focused PCD can be calibrated using a reference pressure approximated from planar hydrophone
measurements, using either the pulse-echo or pitch-catch technique. Additionally, numerical results indicate that both techniques are appropriate to calibrate a variety of other PCD geometries. For the pulse-echo technique, relatively low error between the approximated reference pressure and the PCD-measured pressure can be maintained for the calibration of any PCD with an $f$–number greater than 0.5. To maintain relatively low error for the pitch-catch technique, care must be taken to use a focused source with an equivalent or smaller $f$–number than the PCD. Otherwise, the PCD diameter will exceed the transmit beamwidth and the pressure wave will arrive nonuniformly across its face, resulting in appreciable phase cancellation and associated measurement errors, especially at higher frequencies.

The bandwidth of any calibration is generally dependent on the combined, finite bandwidths of the transmit and receive sensitivities of the transducer pair used for measurements. Both transducer configurations used here resulted in relatively high SNR measurements over a broad bandwidth. Therefore, the PCD receive sensitivities were also determined over a broad bandwidth for both methods, including the pitch-catch technique, even though the source and PCD had different center frequencies of approximately 4.5 and 8.1 MHz, respectively. Considering the bandwidth and precision of measurements made here, both calibration methods were shown to produce approximately the same receive sensitivity for the PCD over a broad range of frequencies and were validated by comparison with hydrophone measurements over a range of frequencies and pressures. These results indicate that both techniques are suitable for calibrating the receive sensitivity of focused, single-element PCDs in order to make absolute
pressure measurements.

Since the PCD and hydrophone directly measure the transmit beam of the source, the acquired signals provide relatively high signal-to-noise ratio (SNR) over a broad frequency range, limited mainly by the combined bandwidth of the transmit-receive transducer pair. In contrast, the bistatic scattering substitution technique employs a scattered wave with amplitude much smaller than the incident wave, resulting in lower SNR and thus potential limitations in useful bandwidth. The rapid decrease in measurement accuracy at higher frequencies that occurs with the bistatic scattering substitution technique does not occur for the pitch-catch substitution method, as long as the focused source employed has an $f$-number equal to or smaller than the PCD $f$-number. Accurate broadband PCD calibrations are feasible with any of the investigated methods, as long as appropriate consideration is given to the frequency range of interest, to transducer geometries, and to scatterer properties for the bistatic scattering substitution method.

### 3.4.3 Pulse-echo calibration technique for unfocused receivers

Numerical results shown here demonstrated that the receive sensitivity of a flat-piston PCD can be accurately determined using a pulse-echo method with the reference pressure approximated from planar hydrophone measurements calculated as the average pressure magnitude to represent the average complex pressure received by a PCD. The maximum disagreement between the hydrophone-measured reference pressure and that measured by the PCD was found to be less than 0.5 dB for various
PCDs, frequencies, or separation distances between the PCD and reflection plate or hydrophone-measured plane. This indicates acoustic measurements can be made in the near- or far-field of a PCD for any frequency and do not require additional compensations for diffraction effects.

Although the difference of up to 0.5 dB in simulated measurements shows that there may be inherent error due to the reference pressure approximation, this value is minimal in comparison to the error that would be contributed from misalignment of the PCD or hydrophone using the average complex pressure. Hence, error using this method is primarily limited to the alignment of the PCD and reflection plate. The error incurred by misalignment in the range direction of the reflection plate and PCD was significant only for low $ka_R$ values. However, for higher frequencies and for larger diameter transducers resulting in higher $ka_R$ values, error was shown to be especially sensitive to the axial angle alignment of the PCD and reference plate. For example, for a frequency and PCD with a $ka_R = 200$, error between aligned and misaligned PCD-measured pressures exceeds 6 dB at as little as a 0.9° misalignment in one axial directions, however at a $ka_R = 50$ the same level of error was not incurred until the axial misalignment exceeded 3°. In addition, since the pulse-echo technique is analogous to a pitch-catch technique consisting of two equal geometry transducers, these results indicate a pitch-catch technique can also be employed for calibrating unfocused transducers, with equivalent accuracy when the transmit and receive transducers are approximately identical.

The pulse-echo technique was conducted for a flat piston transducer over a range of frequencies of 0.07–2.5 MHz ($ka_R = 5–134$). The maximum error in repeated pulse-
echo measurements made by the transducer was compared with the simulated error due to a $1^\circ$ misalignment, illustrating a relatively good agreement and indicating that the uncertainty provided from repeated pulse-echo measurements was likely due to an axial misalignment of a maximum of $1^\circ$. Even with this potential error, the average uncertainty over the calibration bandwidth from repeated pulse-echo measurements was 12.75% despite the rapidly decreasing SNR for frequencies less and greater than the center frequency of 1 MHz. However, this value was substantially less than that contributed by the manufacturer-calibrated hydrophone of 29% on average across the same frequency range. This indicates that this method is repeatable with good accuracy for calibrating flat transducers, but the primary source of uncertainty in the calibration may be dependent on the precision of the manufacturer provided calibration of the hydrophone used for reference measurements.

The sensitivity of the transducer used as a PCD during sonophoresis trials in Chapter 2 was determined based on the measured sensitivity of a second transducer due to the former being unavailable. However, the frequency response, center frequency, and bandwidth were nearly identical between the two transducers, hence it was assumed the difference in receive sensitivities would be proportional to the difference in the amplitude response in pulse-echo measurements. Likewise, the frequency-dependent uncertainty associated with the calibration measurements was assumed the same since it was shown in numerical and experimental results that the error in measurements is dominated by misalignment and the uncertainty dominated by that contributed by the hydrophone used for reference measurements. The
calibration of this latter transducer and the electronics employed for sonophoresis in Chapter 2 enables the potential for quantitative analysis by calculating the cavitation-radiated acoustic pressure, energy or power received by the PCD.

Although the pulse-echo technique was employed similarly to calibrate the receive sensitivity of a focused (Figure 3.11) and unfocused transducer (Figure 3.16), the resulting sensitivities illustrate the differences between these two transducer types when employed as receivers. For example, the available bandwidth of the focused transducer was approximately 20 MHz while for the unfocused transducer the calibration bandwidth was limited to 2.5 MHz. This is due to the SNR of the focused transducer being significantly greater over a large range of frequencies. Additionally, the sensitivity of >15 V/MPa for focused transducer at its center frequency was much greater than that of the unfocused transducer of < 1 V/MPa. Although a focused transducer used as a PCD offers significantly greater SNR and sensitivity in comparison to an unfocused transducer, its geometric focus limits the region of interrogation typically to an area in the azimuth direction on the order of millimeters squared. For the sonophoresis trials conducted in Chapter 2, for example, treated skin samples provided a surface area of 755 mm$^2$, hence an unfocused PCD was chosen in order to interrogate the greater area.

3.4.4 Considerations for accurate receive calibration measurements

Additional factors that may influence PCD receive calibration measurement accuracy include any temperature dependence of measurements and the potential for nonlinear effects. Specifically, erroneous alignment of transducers may be facilitated
by inaccurate temperature measurement of the fluid used as the propagation medium, leading to imprecise time-of-flight estimates that result in inaccurate axial separation distances between the PCD and measurement plane. However, for minor error in temperature measurements, the resulting axial misalignment will be minimal. For example, temperature errors of ±2° C will lead to a ±0.37% maximum difference in the calculation of the speed of sound in water. This calculation error is directly propagated to the alignment of the PCD and measurement plane separation distance. For the calibration of the unfocused transducer conducted in Section 3.2.5, a water temperature measurement error of ±2° C would lead to an axial misalignment of less than ±0.25 mm from the desired 65 mm separation distance. This error in displacement, normalized as \((z_M - z_0)/a_R = 0.02\), is not sufficient to result in significant error even for measurements made within the far field as shown in Figures 3.8(a) and 3.9(a). In addition, due to the large volume of water typically used in calibration measurement systems and the relatively high specific heat of water, any spatiotemporal variations in the temperature of the propagation medium are likely to be small.

Simulated and experimental calibration measurements made in this chapter were conducted under linear acoustics assumptions. That is, small-signal assumptions were not expected to be violated and nonlinear effects were assumed negligible. The assumed negligible effects from nonlinear propagation were made in part due to employing relatively low acoustic intensities. For example in Section 3.2.5, the transmitted on axis pressure at a range distance of \(z = 65\) mm by the unfocused transducer at its center frequency of 1 MHz was 22 kPa, corresponding to an
equivalent pressure, $P_0$, at the source surface of approximately 21 kPa. The shock formation distance in water (with a coefficient of nonlinearity $\beta_0 = 3.5$) for this frequency and initial pressure, calculated as $\bar{z} = \frac{\rho c^3}{(\beta_0 \omega P_0)}$ [Pierce, 1981], is greater than 7 m. Hence, the calibration measurements conducted for the unfocused transducer were made at an axial distance that was on the order of $1/100$ of the shock formation distance, suggesting that effects due to nonlinear propagation were negligible. Although nonlinear effects may influence both the PCD and reference measurements, it is unclear whether the effects would influence both measurements in an equivalent manner or lead to an additional source of error. The contribution to error in calibration measurements of nonlinear propagation should be investigated in future studies; otherwise future calibration measurements should be conducted by employing low intensity measurement conditions at distances much less than the shock formation distance. For quantitative cavitation emission measurements using a calibrated PCD, consideration of nonlinear effects should be made. For example, although emissions from a single cavitation bubble radiating in a linear material may not exhibit nonlinear effects [Collin and Coussios, 2011], nonlinear propagation may influence measurements of emissions from microbubble clouds in tissue [Tang et al., 2010].

As shown in this chapter, the accuracy of calibration measurements is dependent on the alignment of transducers, or the reference plane, or both. Error in axial separation distance may occur due to inaccurate temperature measurements, resulting in erroneous time-of-flight calculations. These errors will likely be relatively small for near field measurements. However, angular misalignment may lead to
greater inaccuracies, especially at higher frequencies and for larger transducer
diameters (Figure 3.8(b)). It is therefore imperative to ensure orthogonality
between the measurement plane or receive transducer surface with the propagating
pressure wave that is being measured, in addition to accurate water temperature
measurements. First, to minimize temperature increases at the receiver surface,
source-transmitted pressure waves can be generated with a low duty-cycle and
relatively low output pressure. In addition, these parameter settings will also
reduce the probability of cavitation activity occurring near the receiver surface and
influences of nonlinear propagation.

Second, because calibration measurements for the pitch-catch and pulse-echo
techniques are implemented in a comparable manner to transmit field calibration
measurements, similar approaches can be made to ensure alignment. Specifically, for
hydrophone measurements, this approach is straightforward, as measurements can
be made and compared for symmetry over multiple axial planes. For alignment of
the PCD with the source transducer using a pitch-catch or pulse-echo technique,
the PCD or reflection plate can be scanned to find the maximum signal strength at
multiple separation distances by conducting an area scan in each plane. Conducting
this alignment at different separation distances will aid in reducing angular
misalignment with the source or reflector. Additionally, the alignment and associated
measurements can be repeated, as done for the measurements conducted in this
chapter, in order to assess the uncertainty due to misalignment.
Chapter 4

Characterization of
cavitation-radiated acoustic power

4.1 Introduction

For many biomedical acoustics applications, passive measurements made using a
single-element transducer as a receiver to monitor scattered or radiated sound from
cavitating bubbles are made as system-dependent measurements. Specifically, the
voltage signal generated by the receive system and used for analysis is influenced
by the frequency response of the acquisition components and the receive sensitivity
of the transducer used as a receiver. This challenge may be overcome by measuring
the absolute radiated or scattered pressure incident on the receiving transducer,
requiring characterization of the acquisition system and the sensitivity of the
transducer on receive using the techniques developed in Chapter 3, a bistatic
scattering substitution method [Collin and Coussios, 2011, Sboros et al., 2005],
or self-reciprocity methods [Carstensen, 1947, Hill and Egle, 1980, Zhang et al.,
2016]. Quantitative measurement approaches have been employed for investigations
in areas such as cavitation dynamics [Collin and Coussios, 2011] and acoustic backscatter measurements [Chen et al., 1997, Sboros et al., 2005].

For monitoring emissions generated by cavitating bubbles, absolute pressure measurements can be made using a calibrated single-element passive cavitation detector (PCD). These measurements can be used to characterize the pressure or acoustic power generated by a single cavitating bubble, if its position is maintained within the focal region of the PCD such that diffraction effects are negated, by compensating the derated measurement for spherical spreading [Collin and Coussios, 2011]. This characterization approach offers a potential technique for system-independent measurements of single bubbles with the ability to characterize the radiated pressure or acoustic power, and the source-strength amplitude of the cavitating bubble.

However, for most cavitation-based therapeutic ultrasound applications, measured pressures emanate from an ensemble of cavitating bubbles for which the spatial distribution at any point in time is not known or readily predicted [Maxwell et al., 2013]. For these measurements, the received pressure is typically less than the expected value due unaccounted diffraction effects that vary with the position and frequency of individual acoustically activated bubbles as well as the geometry of the PCD. Hence, frequency-dependent pressures measured by different PCDs, even when monitoring the same cavitating bubbles, will not be directly comparable. Furthermore, spatially-dependent diffraction effects associated with individual cavitating bubbles cannot be accounted for because emission measurements using a single-element PCD are made without the ability to spatially or temporally resolve
the signal associated with individual bubbles. Hence, estimating the source-strength amplitude and radiated acoustic power of individual cavitating bubbles within an ensemble of unknown distribution is challenging.

The challenge in comparing measurements made between different frequencies or receiving systems is evident in the sonophoresis trials conducted in Chapter 2. The measured emissions made during intermediate- (IFS) and high-frequency sonophoresis (HFS) are not directly comparable because of frequency-dependent variations in the receive system response, PCD sensitivity, and diffraction in the cavitation radiated field. Comparison of emission measurements may allow further investigation into the efficacy of each treatment regime by enabling deeper investigation into the interaction of cavitation with skin. For example, measured changes in skin resistance were shown to be comparable between IFS and HFS in Chapter 2. However, it is not clear if the relationship between the changes in skin resistance and the exposure to cavitation were equivalent for the different treatment regimes. Specifically, identification of the relationship of cavitation-radiated power required to induce permeability changes in skin may be used to better guide future treatments, including the choice of insonation frequency and power. Additionally, these results are not able to be compared with other published results. One approach to circumvent these limitations would be to characterize the radiated acoustic power from cavitation on the skin surface.

The absolute acoustic pressure and associated power measured by a calibrated single-element PCD are subject to diffraction effects dependent on the frequency of emissions, PCD geometry, and the unknown positions of emission-generating
cavitation sources. Here, a method is developed for characterizing an ensemble of emission sources, quantified by the radiated acoustic power per unit area or volume within defined region of interest (ROI). This approach can be conducted without a priori knowledge of the number or spatiotemporal distribution of cavitation events. Included in the following sections is the theory employed for deriving an analytic diffraction-correction factor relating frequency-dependent PCD-measured pressures to the acoustic power radiated by cavitating bubbles within a defined ROI. Simulations were employed to show that the cavitation-radiated acoustic power per unit ROI volume or area can be accurately recovered by diffraction correction of emissions received by focused or unfocused PCDs. Additionally, the diffraction-correction factor associated with the frequencies and configuration employed for sonophoresis in Chapter 2 is calculated. Using this factor with the system calibration values measured in Chapter 3 and emission measurements made in Chapter 2, the radiated acoustic power from cavitation on the skin surface during sonophoresis is characterized. Following this characterization, a direct multi-frequency analysis is conducted between the radiated acoustic power from cavitation on the skin surface and changes in skin resistance between intermediate- (IFS) and high-frequency sonophoresis (HFS).
4.2 Theory

4.2.1 Characterizing the acoustic power radiated by a single cavitating bubble using a single-element receiver

A single cavitating bubble can be modeled as a radially pulsating acoustic source. For a spherical source with small dimensions compared to the wavelength of generated sound, the limit of the pulsating sphere solution at far radial distances becomes that of an acoustic monopole. Hence, the frequency-dependent pressure, $P_i(f)$, at a far-field location, $r$, generated by a single (i.e., $i=1$) cavitating bubble centered at $r_i$, pulsating with radial frequency, $\omega = 2\pi f$, within an isotropic medium of density, $\rho_0$, and sound speed, $c$, can be represented as [Pierce, 1981]

$$P_i(r, f) = -j\omega\rho_0 Q_i(f) \frac{e^{i\omega|r_i-r|}}{4\pi |r_i-r|}, \quad (4.1)$$

where the cavitating bubble is characterized by its frequency-dependent complex source-strength amplitude, $Q_i(f)$, the outward volume rate of fluid flow from the pulsating sphere (units of m$^3$/s). The acoustic intensity associated with the radiated pressure at a field location $r$ is calculated as the magnitude squared of the corresponding complex pressure value at $r$ divided by two times the acoustic impedance as [Blackstock, 2000]

$$I_i(r, f) = \frac{|P_i(r, f)|^2}{2\rho_0 c} \quad (4.2)$$

Because the radiated pressure is spherical in directivity, the total radiated...
acoustic power can be calculated by integrating the radiated acoustic intensity over an enclosing spherical surface, $S_S$, with radius $r_S = |r_i - r|$, the distance from a cavitating bubble centered at $r_i$ to a location $r$ on the enclosing surface. The frequency-dependent acoustic power radiated by a cavitating bubble, $\Pi_{T_i}(f)$, can be calculated in terms of the source-strength amplitude, $Q_i(f)$, by combining Equations 4.1 and 4.2, as [Norton and Karczub, 2003]

$$\Pi_{T_i}(f) = \frac{\omega^2 \rho_0}{8\pi c} |Q_i(f)|^2.$$  \hspace{1cm} (4.3)

Notably, the total acoustic power radiated by a single cavitating bubble is directly proportional to the squared magnitudes of the radial pulsation frequency, $\omega$, and source-strength amplitude, $Q_i(f)$, and is not influenced by its position or phase effects in the radiated pressure field.

In comparison, the acoustic pressure radiated from a cavitating bubble that is received by a phase sensitive transducer is dependent on the bubble location and element geometry. Specifically, the element-received pressure from a cavitating bubble, $\bar{P}_{R_i}(r_R, f)$, is calculated as the radiated complex pressure, $P_i(r, f)$, incident on and spatially averaged over the surface of the receiving element as [Chen et al., 1997]

$$\bar{P}_{R_i}(r_R, f) = \frac{1}{S_R} \int_{S_R} P_i(r, f) dS(r_R),$$ \hspace{1cm} (4.4)

where $r_R = |r_i - r|$ is the distance from a cavitating bubble centered at $r_i$ to a
location, $r$, on the element surface, $S_R$. By combining Equations 4.1 and 4.4, the average incident complex pressure on an element can be expressed in terms of the source-strength amplitude, $Q_i(f)$, as

$$
\bar{P}_{R_i}(r_R, f) = -Q_i(f)\frac{\rho_0 c}{S_R} \frac{j k}{4\pi} \int_{S_R} \frac{e^{jk r_R}}{r_R} dS(r_R),
$$

(4.5)

where $k = \omega/c$. The frequency-dependent transmitted pressure field by a given element, radiating uniformly with unity nominal surface pressure, at the location, $r_i$, of the cavitating bubble can be represented by the Rayleigh integral in the form [Rayleigh, 1945, Mast and Yu, 2005]

$$
P_{\text{Rayl}}(r_i, f) = -\frac{j k}{2\pi} \int_{S_R} \frac{e^{jk r_R}}{r_R} dS(r_R).
$$

(4.6)

Because of reciprocity, the magnitude squared of $P_{\text{Rayl}}(r_i, f)$ in Equation 4.6 represents the relative spatial sensitivity of an element to a cavitating bubble at $r_i$. Using this value, the element-received complex pressure from a single cavitating bubble can be calculated in terms of $P_{\text{Rayl}}(r_i, f)$ and the source-strength amplitude, $Q_i(f)$, by combining Equations 4.5 and 4.6 as

$$
\bar{P}_{R_i}(r_R, f) = \frac{\rho_0 c}{2S_R} Q_i(f) P_{\text{Rayl}}(r_i, f).
$$

(4.7)

In this form, varying diffraction effects in the element-received pressure due to the element geometry, frequency of emissions, and cavitating bubble position can be
attributed to the complex value of $P_{\text{Rayl}}(r_i, f)$. Hence, the received pressure will vary between different sized or positioned receivers, even when monitoring the same cavitating bubble if it is not located within the focal region associated with a given receiver, because of varying diffraction effects.

The acoustic power associated with the element-received pressure from a cavitating bubble is calculated as the magnitude squared of the average incident pressure, $\bar{P}_{R_i}(r_R, f)$, divided by two times the acoustic impedance [Haworth et al., 2017]. This value can be related to the cavitating bubble, expressed in terms of the source-strength amplitude, $Q_i(f)$, as well as the associated Rayleigh integral, $P_{\text{Rayl}}(r_i, f)$, by incorporating the element-received pressure term, $\bar{P}_{R_i}(f)$, from Equation 4.7 as

$$\Pi_{R_i}(f) = \frac{S_R}{2 \rho_0 c} |\bar{P}_{R_i}(f)|^2$$

$$= \frac{\rho_0 c}{8 S_R} |Q_i(f) P_{\text{Rayl}}(r_i, f)|^2.$$  \hspace{1cm} (4.8)

Notably, in comparison to the radiated power in Equation 4.3, the acoustic power received by a transducer element in Equation 4.8 is influenced by diffraction effects from phase cancellation of the cavitation-radiated complex pressure incident on the finite aperture of the element. Hence, the acoustic power received by an element typically provides an underestimate of the acoustic power radiated by a cavitating bubble. In order to recover the acoustic power radiated by a single cavitating bubble from the received value, a unitless diffraction correction factor, $\hat{\Gamma}(f)$, can be derived as the ratio of the radiated acoustic power (Equation 4.3) to the corresponding
measured power (Equation 4.8), as

\[
\hat{\Gamma}(f) = \frac{\Pi_{T_i}(f)}{\Pi_{R_i}(f)} = \frac{S_R}{\pi} \frac{k^2}{|P_{Rayl}(r_i,f)|^2},
\]

(4.9)

where the amplitude squared of the associated Rayleigh integral, \(P_{Rayl}(r_i,f)\), accounts for the relative spatial sensitivity of the receiving element to a cavitating bubble located at \(r_i\). Calculation of this factor can readily be accomplished using known values of the element surface area, \(S_R\), and wavenumber, \(k\), associated with the frequency of emissions, as well as the calculation of the Rayleigh integral, \(P_{Rayl}(r_i,f)\), at the location of a single cavitating bubble and for a given element using established methods [Hasegawa et al., 1986, Ocheltree and Frizzel, 1989, Mast and Yu, 2005, Mast, 2007].

For a single cavitating bubble located at the focal position of an element, the received pressure can simply be adjusted for spherical spreading of the radiated field in order to determine the radiated pressure and acoustic power due to the radiated pressure wave arriving uniformly across the receiver surface [Collin and Coussios, 2011]. Otherwise, the factor \(\hat{\Gamma}(f)\) is required to account for diffraction effects associated with the frequency, cavitating bubble location, and geometry of the receiving element. Specifically, after calculating the factor, \(\hat{\Gamma}(f)\), the radiated acoustic power, \(\Pi_{T_i}(f)\), can be retrieved from the acoustic power measured by a calibrated element, \(\Pi_{R_i}(f)\), by rearranging Equation 4.9 and applying the calculated factor to the measured value. However, this characterization technique requires the
use of a receiving element with known receive sensitivity and is strictly limited to
the case of a single cavitating bubble at a precisely known location.

4.2.2 The total radiated and element-received acoustic power
from an ensemble of cavitating bubbles

For an ensemble of $N$ cavitating bubbles, the radiated complex pressure at a field
location $r$, $P(r,f)$, is equal to the $N$-term summation of the individual radiated
complex pressures (Equation 4.1), $P_i(r,f)$, at that location as

$$P(r,f) = \sum_{i=1}^{N} P_i(r,f). \quad (4.10)$$

Using this pressure value, the acoustic intensity and power radiated by the ensemble
are calculated following the approach employed for a single cavitating bubble in
Equations 4.2 and 4.3, respectively. Specifically, the ensemble-radiated acoustic
intensity at $r$ is proportional to the magnitude squared pressure, $P(r,f)$, at that
position by

$$I(r,f) = \frac{\left|\sum_{i=1}^{N} P_i(r,f)\right|^2}{2\rho_0 c}. \quad (4.11)$$

Additionally, the total radiated acoustic power is calculated by integrating the
ensemble-radiated intensity $I(r,f)$ over an enclosing surface, $S_s$, by

$$\Pi_T(f) = \frac{1}{2\rho_0 c} \int_{S_s} \left|\sum_{i=1}^{N} P_i(r,f)\right|^2 dS(r_s), \quad (4.12)$$
where $r_S = |r - r_i|$ is the distance from the $i$–th cavitating bubble located at $r_i$ to a location, $r$, on the enclosing surface, $S_S$.

The received pressure $\bar{P}_R(f)$ from an ensemble of $N$ cavitating bubbles is calculated as the $N$–term summation of the spatially averaged complex pressures, $\bar{P}_{R_i}(r_R, f)$, as defined in Equation 4.5, incident on the element surface from an individual cavitating bubble. This value can be expressed in terms of the individual source-strength amplitudes, $Q_i(f)$, and associated Rayleigh integrals, $P_{Rayl}(r_i, f)$, by incorporating the received pressure, $\bar{P}_{R_i}(r_R, f)$, from an individual cavitating bubble in Equation 4.7 as

$$\bar{P}_R(f) = \sum_{i=1}^{N} \bar{P}_{R_i}(r_R, f)$$

$$= \frac{\rho_0 c}{2S_R} \sum_{i=1}^{N} Q_i(f) P_{Rayl}(r_i, f).$$

(4.13)

Correspondingly, following Equation 4.8 for a single bubble, the acoustic power received by an element from an ensemble of cavitating bubbles is proportional to the magnitude squared of the cumulative complex pressure incident on and spatially averaged over its surface. Using the average received pressure $\bar{P}_R(f)$ value in Equation 4.13, the received acoustic power from an ensemble can be calculated in terms of the individual source-strength amplitudes $Q_i(f)$ and associated Rayleigh integrals $P_{Rayl}(r_i, f)$ as

$$\Pi_R(f) = \frac{S_R}{2\rho_0 c} |\bar{P}_R(f)|^2$$

$$= \frac{\rho_0 c}{8S_R} \left| \sum_{i=1}^{N} Q_i(f) P_{Rayl}(r_i, f) \right|^2.$$

(4.14)
The calculation of the received pressure and power in Equations 4.13 and 4.14, respectively, assumes cavitating bubble of known locations, \( r_i \), within the ensemble. However, cavitation emission measurements made using a single-element receiver are typically acquired without \textit{a priori} knowledge of the distribution of cavitating bubbles, that may occur with spatiotemporal stochasticity and cannot be readily predicted [Maxwell et al., 2013]. Without precise knowledge of the cavitating bubble locations, it becomes challenging to characterize individual bubbles within an ensemble in part because of the inability to relate the cavitation-radiated power to that measured by the receiving element analytically in Equations 4.12 and 4.14, respectively. This is in part due to the inability to distinguish received signals from individual cavitating bubbles and precisely correct for diffraction effects associated with individual cavitating bubbles within the ensemble. Therefore, the approach employed for characterizing the average power radiated by a single bubble at a known location using calibrated emission measurements cannot be directly extended to the case of multiple cavitating bubbles at unknown locations.

4.2.3 Characterizing the acoustic power radiated by an ensemble of cavitating bubbles using a single-element receiver

One potential system-independent approach to characterizing an ensemble of cavitating bubbles at unknown locations would be to relate the average acoustic power received by a calibrated PCD to the average acoustic power radiated by cavitation per unit measure of a region of interest (ROI), where the ROI is defined as the region containing the bubbles of interest. This approach can be accomplished
without \textit{a priori} knowledge of the cavitating bubble characteristics by assuming an ensemble can be modeled as a wide sense stationary random process consisting of cavitating bubbles that occur with stochastic independence.

First, an ensemble of cavitating bubbles is modeled as a wide sense stationary random process such that the ensemble mean of random variables characterizing stochastic cavitation events are assumed to be unchanging with respect to time over a given observation period. Hence, within a given time period, the expected value of these characteristics can be represented statistically by corresponding time averaged values realized over multiple observation. Under this assumption, the time average of the acoustic power radiated by an ensemble can be represented by the expected value of Equation 4.12 and expanded as

\[
\langle \Pi_T(f) \rangle = \frac{1}{2} \rho_0 c \int_S \left| \sum_{i=1}^{N} P_i(r_S, f) \right|^2 dS(r_S)
\]

\[
= \frac{1}{2} \rho_0 c \int_S \left( \sum_{i=1}^{N} P_i(r_S, f)^* \sum_{j=1}^{N} P_j(r_S, f) \right) dS(r_S)
\]

\[
= \frac{1}{2} \rho_0 c \int_S \left( \sum_{i=1}^{N} |P_i(r_S, f)|^2 + \sum_{i \neq j}^{N} P_i(r_S, f)^* \sum_{j=1}^{N} P_j(r_S, f) \right) dS(r_S),
\]

(4.15)

where for any given variable \(a\), the complex conjugate of the \(i\)–th element is indicated as \(a_i^*\) and the expected value is indicated as \(\langle a \rangle\). Likewise, the time average of the acoustic power received by a given PCD from an ensemble of cavitating bubbles can
be represented by the expected value of Equation 4.14 and expanded as

\[
\langle \Pi_R(f) \rangle = \frac{\rho_0 c}{8S_R} \left( \sum_{i=1}^{N} |Q_i(f)|^2 |P_{Rayl}(r_i, f)|^2 \right) + \frac{\rho_0 c}{8S_R} \left( \sum_{i \neq j} Q_i(f)^* P_{Rayl}(r_i, f)^* Q_j(f) P_{Rayl}(r_j, f) \right). \tag{4.16}
\]

Because the physical properties of the cavitating bubbles do not vary with respect to time over a given observation period, due to the ensemble being ergodic in the wide sense, the mean radiated pressure, and by extension the mean acoustic power, received at a field location \( r \) will also not vary with respect to time over the same observation period. That is, the radiated and received acoustic powers in Equations 4.15 and 4.16, respectively, are assumed to be mean-stationary over the duration of a given temporal observation period. In practice, this temporal observation period over which stationarity is expected corresponds to the duration of the time-domain signal used to estimate average power spectra.

Second, cavitating bubbles within an ensemble can be modeled as stochastically independent events, where the realization of one cavitating bubble does not affect the probability of occurrence or probability distribution of any other. Therefore, the random variables characterizing stochastic cavitation events are uncorrelated and, by definition, the covariance between random variables associated with different bubbles is zero. Specifically, this assumption is valid for an ensemble of cavitating bubbles that pulsate as an ensemble of randomly incoherent sources such that the covariance is zero between the radiated pressures, \( P_i(r, f) \), received at any given field location, \( r \), from any pair of cavitating bubbles. However, this assumption
can also be extended to instances in which the ensemble pulsates with constant phase, for example when an ensemble of bubbles is excited by a coherent source. For an ensemble of randomly distributed cavitating bubbles that are pulsating with constant phase, the propagation path length from each bubble to a given field point, \(r\), varies randomly such that the time delay and resulting phase difference between the pressures received at \(r\) from different cavitating bubbles will also vary randomly. Therefore, the resulting pressure field at a location \(r\) will be the summation of random phased pressures, even for pressures that originate from bubbles that oscillate with constant phase. Alternatively, for instances in which the ensemble pulsates with constant phase and the spatial distribution is much less than a wavelength, the pressures received from individual cavitating bubbles will arrive coherently. For this instance, because of the limited spatial extent over which cavitation occurs and the coherence among the cavitating bubbles, the collection of bubbles can instead be modeled as a single acoustic source instead of as an ensemble.

Considering the assumption of independence for an ensemble, the expected value of the second summation term of the integrand in Equation 4.15 goes to zero because the complex pressure, \(P_i(r_S, f)\), from a given bubble and the complex conjugate of the pressure, \(P_j(r_S, f)^*\), from any other bubble that is received at a given field location, \(r\), are uncorrelated. After rearranging the order of integration and summation, the remainder of 4.15 can be equivalently represented as the average of the incoherent summation of acoustic power radiated by individual cavitating bubbles, \(\Pi_{T_i}(f)\), described in Equation 4.3. Hence, by combining Equations 4.3 and 4.15 and dividing by the area or volume, \(W\), of a defined ROI, the average acoustic
power radiated per unit measure of a given ROI can be approximated in terms of the expected value of the magnitude-squared source-strength amplitude, \( \langle |Q(f)|^2 \rangle \), of the ensemble as

\[
\frac{\langle \Pi_T(f) \rangle}{W} \approx \frac{1}{2\rho_0 c W} N \left\langle \sum_{i=1}^{N} \left( \int_{S_S} |P_i(r_S, f)|^2 dS(r_S) \right) \right\rangle \\
\approx k^2 \frac{\rho_0 c}{8\pi} N \langle |Q(f)|^2 \rangle .
\]

(4.17)

Notably, for a given ROI, the acoustic power radiated by an ensemble increases linearly with the number of bubbles, \( N \), and with the square of the frequency, \( k \), of generated emissions.

From Equation 4.7, the received pressure from a cavitating bubble is proportional to the product of its source-strength amplitude, \( Q_i(f) \), with the associated Rayleigh integral, \( P_{Rayl}(r_i, f) \). Therefore, the summand of the second term in Equation 4.16 is equivalent to the product of the received pressures from different cavitating bubbles and this term goes to zero because the covariance between these values for any pair of bubbles is zero. Following this assumption, the expected value of the acoustic power received by an element as calculated in Equation 4.16 can be approximated in terms of the expected value of the magnitude-squared source-strength amplitude, \( \langle |Q(f)|^2 \rangle \), of the ensemble as

\[
\langle \Pi_R(f) \rangle \approx \frac{\rho_0 c}{8S_R} N \left\langle \sum_{i=1}^{N} |Q_i(f)|^2 |P_{Rayl}(r_i, f)|^2 \right\rangle \\
\approx \frac{\rho_0 c}{8S_R} N \langle |Q(f)|^2 \rangle \frac{1}{N} \sum_{i=1}^{N} |P_{Rayl}(r_i, f)|^2 .
\]

(4.18)
Notably, in comparison to the total radiated power, the expected value of the received acoustic power includes the average magnitude-squared Rayleigh integral of the $N$ cavitating bubbles within an ensemble. However, to this point, in order to calculate the total radiated or received acoustic power, the positions, $r_i$, number, $N$, and source-strength amplitude, $Q_i(f)$, of the individual cavitating bubbles within an ensemble are required to be precisely known.

To extend this calculation to multiple bubbles at unknown locations, the assumption that the ensemble can be modeled as a wide-sense stationarity process can be employed such that the position, $r_i$, of individual cavitating bubbles can be statistically represented on average by a spatially uniform distribution within the surface or volumetric dimensions, $W$, of the ROI. Hence, the calculation of the received acoustic power is extended to multiple cavitating bubbles at unknown positions by varying the presumed location, $r_i$, of bubbles over a uniform grid within the defined ROI. Following this approach, the relative spatial sensitivity of a receiving element to cavitating bubbles within a given ROI can be estimated as the spatial average of the magnitude squared value of the Rayleigh integral calculated at uniformly discrete locations, $r$, within the defined ROI. Using this calculation, the time-averaged acoustic power incident on an element in Equation 4.18 can be approximated as

$$\langle \Pi_R(f) \rangle \approx \frac{\rho_0 c}{8 \pi R} \left( \langle |Q(f)|^2 \rangle \right) \frac{N}{W} \int_W |P_{Rayl}(r, f)|^2 dW, \tag{4.19}$$

where the average spatial sensitivity to cavitating bubbles at precise locations
calculated in Equation 4.18 is now approximated by the average spatial sensitivity throughout a given ROI.

Using the ratio of Equations 4.17 and 4.19, a diffraction-correction factor, \( \Gamma(f) \), (with units of acoustic power per unit measure of \( W \)) can be derived relating the time-averaged acoustic power measured by a calibrated element, by extension of its measured pressure via Equation 4.14, to the time-averaged total cavitation-radiated acoustic power within a defined ROI as

\[
\Gamma(f) = \frac{\langle \Pi_T(f) \rangle / W}{\langle \Pi_R(f) \rangle} = \frac{S_R}{\pi} \frac{k^2}{\int_W |P_{Rayl}(r,f)|^2 dW},
\] (4.20)

Notably, unknown characteristic quantities of the ensemble of cavitating bubbles, such as the number, \( N \), and expected value of the ensemble magnitude-squared source-strength amplitude, \( \langle |Q_i(f)|^2 \rangle \), are factored out from this calculation and the precise positions, \( r_i \), of cavitating bubbles do not need to be precisely known. Furthermore, this factor consists of known quantities such as the wavenumber, \( k \), and element surface area, \( S_R \), and only requires calculation of the Rayleigh integral for the corresponding PCD over the ROI, which can be conducted using established methods for PCD elements of various geometry [Hasegawa et al., 1986, Ocheltree and Frizzel, 1989, Mast and Yu, 2005].

After calculation of the correction factor in Equation 4.20, the time average of the radiated power per unit area or volume, \( \langle \Pi_T(f) \rangle / W \), of a ROI with dimensions, \( W \), can be characterized by applying \( \Gamma(f) \), calculated for the given PCD, ROI, and
frequency to the time-averaged acoustic power $\langle \Pi_R(f) \rangle$ measured by a calibrated PCD as

$$\frac{\langle \Pi_T(f) \rangle}{W} = \Gamma(f) \langle \Pi_R(f) \rangle.$$ (4.21)

Recalling that $\langle Pi_R(f) \rangle$ can be determined from the measured pressure incident on the element surface (Equation 4.14), this approach requires the receiving element to be calibrated in order to relate the measured voltage to the received pressure. Hence, by extension, this approach enables the acoustic power radiated by cavitation in a defined region of interest to be directly characterized from the system-dependent voltage generated by a PCD receive system. This approach is employed without precise knowledge of the cavitating bubble characteristics by using the factor derived in Equation 4.20 to correct measured pressures by a calibrated element (see Chapter 3) and using the signal processing techniques derived in the following sections.

### 4.2.4 Quantitative signal processing for acoustic measurements by single-element receivers

The generalized analysis discussed in this section considers analytic signals generated or received by a single-element transducer, employed as a passive cavitation detector (PCD). These signal processing techniques are employed for quantitative characterization of the measured acoustic power and pressure from cavitation. A pedagogical explanation of equivalent measurements made using an array of elements is provided by Haworth et al. [Haworth et al., 2017].

In previous studies employing passive cavitation detection using a single-element receiver as a PCD, the quantification of cavitation activity has been limited
to spectral analysis of the time-domain voltage signal, \( x_{\text{sys}}(t) \), generated by the receiving system to represent the average incident pressure, \( \bar{p}_R(t) \), received on the active element. Typically, the strength of signals measured by the receive system and the distribution in frequency, specifically for quantifying spectral components characteristic of different cavitation activities, are analyzed by calculating the energy density spectrum (EDS; with units of squared volts-seconds per unit frequency \( \text{V}^2\text{s}/\text{Hz} \)) of \( x_{\text{sys}}(t) \) as the magnitude squared of its Fourier transform \( X_{\text{sys}}(f) \).

The total electrical energy over all frequencies for this signal can be calculated by integrating the EDS over all frequencies as

\[
E_{\text{sys}} = \int_{-\infty}^{\infty} |x_{\text{sys}}(t)|^2 \, dt = \int_{-\infty}^{\infty} |X_{\text{sys}}(f)|^2 \, df, \tag{4.22}
\]

where, by Parseval’s theorem, the Fourier transform is unitary in that no energy is lost between that of a real continuous voltage signal, \( x_{\text{sys}}(t) \), and its Fourier transform, \( X_{\text{sys}}(f) \), assuming complex conjugate symmetry of the signal such that

\[
|X_{\text{sys}}(f)| = |X_{\text{sys}}^*(-f)|.
\]

For real measurements, the bandwidth of the receiver and associated system electronics is often limited. Furthermore, the measured signal may be additionally filtered to eliminate spectral components with low signal-to-noise ratio or of no relevance to cavitation activity, resulting in a bandlimited signal used for analysis. For these signals, the total energy \( E_{\text{sys}} \) (units of squared volts) of the system-measured voltage signal over a frequency band \( f_1 < f < f_2 \) of interest is calculated
by integrating the EDS of the spectral component within the passband as

\[ E_{\text{sys}}(f_1; f_2) = \int_{f_1}^{f_2} |X_{\text{sys}}(f)|^2 df. \]  

(4.23)

For a continuous signal generated by a stationary random process, such as cavitation activity, the power density spectrum (PDS; with units of squared volts per unit frequency) can be calculated as the average EDS over a truncated time period of finite duration \( T \) to represent that of the continuous-time signal. The average power (units of squared volts) of the truncated and bandlimited electrical signal measured by the system is calculated as the integrated PDS of the spectral components within the passband of \( f_1 < f < f_2 \) by

\[ \Pi_{\text{sys}}(f_1; f_2) = \int_{f_1}^{f_2} \frac{1}{T} |X_{\text{sys}}(f)|^2 df. \]  

(4.24)

For cavitation detection, the energy \( E_{\text{sys}}(f_1; f_2) \) or power \( \Pi_{\text{sys}}(f_1; f_2) \) of the electrical signals measured by the system is commonly used to quantify cavitation activity, providing results in units of \( V^2 \cdot s \) or \( V^2 \), respectively. However, these measurements are not commensurable among different receiving systems due in part to dimensional inhomogeneities. In addition, the measured voltage, \( x_{\text{sys}}(t) \), is typically acquired as a system-dependent measurement and therefore does not directly represent the average cavitation-radiated pressure, \( \bar{p}_R(t) \), incident on the receiver. Hence, the electrical signal energy \( E_{\text{sys}}(f_1; f_2) \) and power \( \Pi_{\text{sys}}(f_1; f_2) \) values do not directly represent the corresponding acoustic values associated with the
observed cavitation events.

As shown in Chapter 3, the Fourier transform of the voltage, $x_{sys}(t)$, measured by the receiving system is related to the Fourier transform of the average complex pressure, $\bar{p}_R(t)$, received by the transducer element by a complex system calibration factor, $M_{sys}(f)$, as

$$\bar{p}_R(f) = \frac{X_{sys}(f)}{M_{sys}(f)} = \frac{X_{sys}(f)}{M_R(f) G_{sys}(f)},$$  \hspace{1cm} (4.25)

where $M_{sys}(f)$ is the product of the receive sensitivity of the receiver, $M_R(f)$, (with units of volts per unit pressure) and the frequency response of the system electronics $G_{sys}(f)$ (a dimensionless transfer function). Using the system calibration factors with the addition of scalar components to obtain proper dimensionality, the acoustic energy and power incident on an element can be calculated from the system-generated electrical signal $x_{sys}(t)$ using an analogous approach to that employed for the corresponding electrical values calculated in Equations 4.23 and 4.24. The acoustic energy (units of Joules) received by the element is calculated by integrating the squared magnitude of the average pressure incident on the element (Equation 4.25) times the surface area of the element, $S_R$, and divided by the acoustic impedance over a frequency band $f_1 < f < f_2$ as

$$E_R(f_1; f_2) = \int_{f_1}^{f_2} \frac{S_R}{\rho_0 c} \left| \frac{X_{sys}(f)}{M_R(f) G_{sys}(f)} \right|^2 df,$$  \hspace{1cm} (4.26)

where $\rho_0$ and $c$ are the density and sound speed of the medium, respectively, and the integrand is the EDS of the spatially averaged instantaneous pressure, $\bar{p}(t)$, incident on the element. Likewise, using the same physical scalars, the acoustic power (units
of Watts) in a frequency band \( f_1 < f < f_2 \) is calculated by integrating the PDS, the average EDS over a truncated time period of finite duration \( T \), within the passband by

\[
\Pi_R(f_1; f_2) = \int_{f_1}^{f_2} \frac{1}{T \rho_0 c} \left| \frac{X_{sys}(f)}{M(f) G_{sys}(f)} \right|^2 df.
\]  \tag{4.27}

The acoustic energy \( E_R(f_1; f_2) \) and power \( \Pi_R(f_1; f_2) \) provide proper physical units associated with the acoustic values measured by the receiver, in units of Joules and Watts, respectively. Additionally, the PCD-received acoustic power \( \Pi_R(f_1; f_2) \) value calculated from the electrical signal here is equivalent that calculated analytically for a single cavitating bubble (Equation 4.8) or an ensemble of cavitating bubbles (Equation 4.19). For discretized signals, additional factors are required for compensation such those derived in the following section.

### 4.2.5 Analysis of received acoustic power from discretized emission measurements by a single-element receiver

In practice, the PCD-received pressure and system-generated voltage signals are digitized for acquisition. For these signals, the element received energy \( E_R(f_1; f_2) \) and power \( \Pi_R(f_1; f_2) \) are calculated from the system-measured electrical signal in the same manner as in Equations 4.26 and 4.27 with the addition of factors accounting for discretization of the signal and processing such as zero padding and windowing [Haworth et al., 2017].

Upon an element receiving cavitation-radiated pressures, the receiving system generates a continuous voltage signal. This signal is reduced to a discrete signal and recorded by the receiving system by uniformly sampling the continuous-time voltage...
a number, $J$, times sequentially over the finite-duration, $T$, of the measurement at a rate of $f_s = J/T$. The sequence of time measurements is taken uniformly over a temporal integer index set of $j = 1 \rightarrow J$ at discretized time points of $t_j = j/f_s$. The corresponding Fourier transform of the discretized time-signal voltage, $x_{sys}[j]$, represented as $X_{sys}[l]$, is discretized with the same number of samples, $L$, as the temporal discretization and over an integer index set of $l = 1 \rightarrow L$ and where square brackets indicate that the signal is discretized. Hence, frequency values of the discretized set are centered at $f_l = l \cdot f_s/L$ with a frequency bin width of $\Delta f_l = f_s/L$.

For discretized signals, the system calibration factor employed for continuous signals in Equation 4.26 and 4.27 is required to be sampled over the same set of frequencies $f_l$ as the discretized system-measured voltage. Using the discretized values, and assuming the entire acoustic signal is finite in duration and contained within the measurement period $T$, the total acoustic energy (units of J) incident on the receiver in a frequency band $f_{l_1} \leq f_l \leq f_{l_2}$ is calculated by summing the spectral components over the corresponding set of frequency indices $l_1 \leq l \leq l_2$ as [Haworth et al., 2017]

$$E_R[l_1; l_2] = \sum_{l=l_1}^{l_2} \left( \frac{S_R}{\rho_0 c} \frac{1}{|w|^2 f_s^2} \left| \frac{X_{sys}[l]}{M_{sys}[l]} \right|^2 \right) \Delta f_l. \quad (4.28)$$

For this calculation the term in parentheses is the energy density spectrum (EDS; with units of J/Hz) of the instantaneous pressure measured by the element, the $f_s$ term accounts for the discretization of $X_{sys}[l]$, the applied time-domain window function, $w$, is accounted for by its mean squared amplitude value, and the additional
physical scalars account for the dimensional transformation of the voltage signal to
acoustic values of pressure and energy by extension.

Likewise, using the same physical scalars and interrogating the same discretized
frequency band $f_{l_1} \leq f_l \leq f_{l_2}$, the acoustic power (units of W) incident on the PCD
is calculated by summing the average EDS over the measurement duration $T$ as
[Haworth et al., 2017]

$$
\Pi_R[l_1; l_2] = \sum_{l=l_1}^{l_2} \left( \frac{S_R}{\rho_0 c T |w|^2 f_s^2} \left| \frac{X_{sys}[l]}{M_{sys}[l]} \right|^2 \right) \Delta f_l, \quad (4.29)
$$

where the term in parentheses is the acoustic power density spectrum (PDS; with
units W/Hz).

4.2.6 Characterizing the cavitation-radiated acoustic power
using discretized emission measurements with a single-
element receiver

For instances when a single positive frequency at a discretized bin value of $f_l$ is
considered where $|X[l]| = |X[-l_1]| = |X[l_2]|$, the received acoustic power calculated
in Equation 4.29 from the system acquired voltage, $X_{sys}(f_l)$, can be evaluated at the
frequency index $l_2$ only, multiplied by a factor of 2 due to symmetry, and used to
represent the power value $\Pi_R(f_l)$ within the frequency bin, $f_l$. Using this measured
value, the radiated acoustic power per unit area or volume of the ROI at frequency,$f_l$, can be estimated using the calculated value of $\Gamma(f_l)$ in Equation 4.20, and by
combining Equations 4.21 and 4.29, in full form as

\[
\frac{\langle \Pi_T(f_i) \rangle}{W} = \Gamma(f_i) \langle \Pi_R(f_i) \rangle = \Delta f_i \frac{\langle |X_{sys}(f_i)|^2 \rangle}{T f_s^2 |w|^2 |M_R(f_i)G_{sys}(f_i)|^2} \frac{8\pi S_R^2}{\rho_0 c^3 f_i^2} \int_w|P_{Rayl}(f_i, r)|^2 dw.
\]

(4.30)

The first term in Equation 4.30 accounts for the discretization of the measured signal. The denominator of the second term consists of calibration factors enabling the conversion of the measured voltage amplitude to the measured pressure amplitude over the element surface. These calibration factors can be determined for a single-element receiver following the methods provided in Chapter 3. The numerator, \(\langle |X_{sys}(f_i)|^2 \rangle\), is the time-average of the magnitude squared voltage generated, discretized, and acquired by the receiving system. The third term consists of physical scalars enabling the conversion of the measured acoustic pressure to power. Finally, the last term accounts for on-average diffraction effects associated with cavitating bubbles throughout the ROI and enables the transformation of the measured acoustic power to the acoustic power radiated by cavitating bubbles within the area or volume of the ROI.

### 4.3 Methods

#### 4.3.1 Correction factor calculation for a volumetric region of interest

The region of interest (ROI) can be defined as the area or volume within a test configuration over which cavitation nuclei are isolated, activated, and emanating acoustic emissions as well as contributing to the overall therapeutic bioeffect.
Characterization of the ROI dimensions may be determined by physical boundaries, by the transmit beam parameters including experimentally determined [Gruber et al., 2014] or estimated [Holland and Apfel, 1989, Bader and Holland, 2012] cavitation threshold values, or by a combination of these factors. Consistent definition of the ROI is required for direct comparisons of emission measurements, which may vary based on the configuration and experiment being considered.

Here, the effect of varying ROI dimensions for different configurations on the calculation of the correction factor $\Gamma(f)$ was investigated. Two configurations were considered, providing an area and volume as the ROI. For a volumetric ROI, a cylindrical vessel was constructed in simulation with a circular diameter of 3 mm to provide comparable dimensions to those employed for experiments commonly conducted within blood vessels [Hitchcock et al., 2011] or cylindrical tubing used to represent blood vessels [Miller et al., 2001, Haworth et al., 2016]. First, the ROI dimensions were bound within the physical boundaries of the simulated cylindrical vessel. Second, the axial length, $x$, of the simulated vessel was varied to provide a range of cylindrical ROI volumes of 2–105 mm$^3$. For this configuration, illustrated in Figure 4.1(a), two transducers were simulated as PCDs and each was configured such that the length, $x$, of the vessel was parallel to the PCD surface. A focused transducer with an $f$-number of 1.5 and circular radius, $a_R$, of 10 mm ($S_R = 323$ mm$^2$) was simulated such that the cylindrical ROI was centered at a range distance, $z$, equal to its focal length of 30 mm. Additionally, an unfocused transducer of equal circular radius ($S_R = 314$ mm$^2$) was simulated as a second PCD. For the unfocused PCD, the cylinder was positioned within the near field at a range distance, $z$, of 35
Figure 4.1: (a) Configuration illustrating the transducer alignment replicated in simulation for a focused and unfocused passive cavitation detector (PCD) and a cylindrical region of interest (ROI). Cross sectional view of the relative spatial sensitivity of a (b) focused and (c) unfocused PCD for a normalized frequency of $ka_R = 200$. A representative ROI with an axial length of 8 mm and cylindrical volume of 56.5 mm$^3$ is outlined in the box within each PCD field.

The relative spatial sensitivity within each volumetric ROI was calculated in MATLAB using an exact series expansion method for the focused [Hasegawa et al., 1986] and unfocused [Mast and Yu, 2005] PCD over a range of normalized frequencies $ka_R$ of 5–450. Shown in Figures 4.1(b) and 4.1(c) are representative dB-scaled maps.
of the relative spatial sensitivities of the focused and unfocused PCDs, respectively, where the value at each position is the frequency-, PCD-, and spatial-dependent normalized and amplitude squared solution to the Rayleigh integral. These spatial sensitivity maps are shown for a \( ka_R \) of 200 for each PCD through the central plane \((y = 0)\) of the vessel. The ROI for these examples is outlined by a box within each figure and is shown for a representative ROI volume of 56.5 mm\(^3\). This figure illustrates the more uniform spatial sensitivity provided by the unfocused PCD over the same ROI, as well as the greater relative sensitivity provided by the focused PCD at the center of this ROI. Using the calculated fields, the associated correction factor, \( \Gamma(f) \), was calculated for each PCD, ROI, and frequency of interest following Equation 4.20. For example, the factor associated with the representative ROI and frequency illustrated in Figures 4.1(b) and 4.1(c) would be calculated in part by integrating the amplitude squared of the values within the specified ROI for either PCD.

4.3.2 Correction factor calculation for a planar region of interest

The configuration shown in Figure 4.2(a) was replicated in simulation to model that used during sonophoresis trials in Chapter 2. This configuration consisted of a flat piston PCD of a circular radius \( a_R = 12.7 \) mm located at a central distance of 4.5 cm below a circular and planar ROI, representing the treated skin surface, facing the ROI at a 45° grade. Because cavitation activity was assumed to be isolated at or near the skin surface during sonophoresis, the ROI for this configuration was simulated as planar, circular area to represent skin surface areas, \( S_{Skin} \), over a range of 10–315
Figure 4.2: (a) Configuration illustrating the transducer alignment replicated in simulation to replicated that used during sonophoresis. The PCD was located at a distance of 4.5 cm and at a 45° grade below the skin surface. The region of interest (ROI) for all simulations was considered as a circular plane on the skin surface. (b) A representative map of the spatial sensitivity over the surface of skin for a PCD and frequency with a $ka_R = 200$. A representative circular ROI with a 5.5 mm circular radius and surface area of 95 mm$^2$ is bounded by a solid line.

The relative spatial sensitivity of the PCD was simulated by calculating in MATLAB (v8.4, MathWorks, Natick, MA) the Rayleigh integral on the skin surface using an exact series expansion method [Mast and Yu, 2005] for a range of normalized frequencies $ka_R$ of 5–450 for each ROI.

The correction factor, $\Gamma(f)$, was calculated for each ROI and $ka_R$ value of interest following Equation 4.20. To illustrate this calculation, the spatial sensitivity of a PCD on the skin surface was calculated and is shown in Figure 4.2(b) for a $ka_R$ value of 200, where the value mapped at each position is the frequency- and spatial-
dependent solution to the Rayleigh integral for this PCD. The solid line indicates the margins of a representative ROI with a 5.5 mm circular radius and a skin surface area of 95 mm$^2$. For this representative configuration, the field sensitivity of the PCD is integrated over all points within the ROI boundaries. The correction factor $\Gamma(f)$ for this representative configuration would then be calculated following Equation 4.20 as the square of the $ka_R$ value divided by the integrated amplitude squared of the Rayleigh integral calculated at each position residing within the defined ROI boundaries.

### 4.3.3 ROI definition and correction factor calculation for sonophoresis

Similar to the calculation over a ROI consisting of an arbitrary skin surface area in the previous section, the correction factor $\Gamma(f)$ was also calculated for the specific PCD geometry (12.7 mm circular radius, $S_R = 507$ mm$^2$) and subharmonic frequencies of interest ($ka_R = 10.8$ and 50.3) employed for the sonophoresis trials conducted in Chapter 2. For these calculations, the configuration shown in Figure 4.2 was replicated in simulation and the ROI dimensions were defined using two different approaches. First, the ROI was defined as the skin surface area of the entire 15.5 mm circular radius sample exposed to sonophoresis in order to encompass all positions on the skin surface in which cavitation could occur. This method was employed because the resistivity of skin is an intrinsic quantity measured over the entire sample and provides equal ROI dimensions among all the various treatment regimes. This method is referred to hereafter as the ‘unweighted’ method as every position on the skin surface was considered, equally weighted by a factor of unity.
Second, the ROI was characterized based on the transmit beam and calculated cavitation threshold pressure values in order to match the region in which cavitation activity was most likely to occur. Hence, the ROI dimensions varied based on the insonation frequency and applied pressure on the skin surface. Because the sonophoresis trials utilized relatively long-duration pulses of 1 second, resonant-sized bubbles were assumed to have formed via coalescence [Bader et al., 2015]. Following the formalism utilized in the Cavitation Index [Bader and Holland, 2012], the peak negative pressure required to initiate subharmonic emissions from resonant sized bubbles in water for each exposure condition was calculated. Next, using the axial profile of the pressure field of each treatment transducer characterized in Chapter 2, the region within the transmit beam on the skin surface that exceeded the corresponding pressure threshold value of the Cavitation Index was used to characterize the boundaries of the ROI. This method is referred to hereafter as the ‘weighted’ method, where integration was performed only over skin surface positions within the ROI defined by the Cavitation Index.

The ROI definitions employed for sonophoresis are illustrated in Figures 4.3 and 4.4 for intermediate- (IFS, \( f_0 = 0.410 \) MHz) and high-frequency sonophoresis (HFS \( f_0 = 2.0 \) MHz), respectively. Shown in Figures 4.3(a) and 4.4(a) are the pressure profiles of the transmit beam on the skin surface for both acoustic powers employed for IFS (0.79 and 1.68 W) and HFS (8.44 and 21.7 W), respectively. The dashed horizontal line in each represents the calculated pressure threshold to induce stable cavitation of resonant sized bubbles based on the Cavitation Index [Bader and Holland, 2012]. The region of the transmit beam on the skin surface for each
Figure 4.3: (a) The axial profile of the acoustic pressure of the transmit transducer used for IFS ($f_0 = 0.41$ MHz) at the skin surface. Each solid line represents that associated with the different acoustic power outputs (APO; 0.79 and 1.68 W) employed for IFS. The dashed line indicates the pressure threshold to initiate subharmonic emissions from resonant sized bubbles, determined from the Cavitation Index. (b) The spatial sensitivity of the PCD on the skin surface for the subharmonic frequency of IFS ($k a_R = 10.8$). Solid lines bound regions of interest (ROI) of corresponding transmit APOs and the dashed line bounds the dimensions of the skin sample exposed to IFS.

Insonation parameter that exceeds this pressure threshold was considered as the ROI based the weighted method. Shown in Figures 4.3(b) and 4.4(b) are maps of the relative spatial sensitivity of the PCD on the skin surface for a $k a_R$ of 10.8 and 50.3, corresponding to the subharmonic ($f_0/2$) of the insonation frequencies ($f_0$) employed for IFS and HFS, respectively. Lines plotted in each figure correspond to the bounded regions associated with the various ROI definitions for each. The two solid lines correspond to the regions defined by the weighted method for each exposure condition. Of these lines, the inner-most solid line corresponds to the ROI
Figure 4.4: (a) The axial profile of the acoustic pressure of the transmit transducer used for HFS ($f_0 = 2.0$ MHz) at the skin surface. Each solid line represents that associated with the different acoustic power outputs (APO; 8.44 and 21.7 W) employed for HFS. The dashed line indicates the pressure threshold to initiate subharmonic emissions from resonant sized bubbles, determined from the Cavitation Index. (b) The spatial sensitivity of the PCD on the skin surface for the subharmonic frequency of IFS ($ka_R = 50.3$). Solid lines contour regions of interest (ROI) of corresponding transmit APOs and the dashed line contours the boundaries of the skin sample exposed to HFS.

of the lower acoustic power, and the outer solid line corresponds to ROI of the higher acoustic power employed for each insonation frequency. Specifically, under this definition, Figures 4.3 and 4.4 show that higher insonation powers result in greater ROI dimensions for this configuration. The dashed line in each figure corresponds to the entire skin surface exposed to sonophoresis for a sample with a circular radius of 15.5 mm. The correction factor, $\Gamma(f)$, associated with each ROI was calculated following previous methods and Equation 4.20. These values, in addition to the average Rayleigh integral and associated ROI dimensions, $W$, on the skin surface.
Table 4.1: Summary of normalization parameters for IFS and HFS subharmonic \((k a_R = 10.8 \text{ and } 50.3, \text{ respectively})\) normalization. Listed are the dimensions of the ROI skin surface area \(w\), spatial average of the amplitude squared Rayleigh integral \(|P_{Rayl}(f)|^2\) over the ROI, and the normalization value \(\Gamma\) for each ROI definition and treatment regime.

| Treatment | ROI definition method | \(w\) (mm\(^2\)) | \(|P_{Rayl}(f)|^2\) | \(\Gamma\) (mm\(^{-2}\)) |
|-----------|----------------------|------------------|-----------------|-----------------|
| IFS       | Weighted (APO = 0.79 W) | 30.3             | 1.75            | 2.2             |
|           | Weighted (APO = 1.68 W) | 100.3            | 1.66            | 0.70            |
|           | Unweighted            | 754.8            | 0.55            | 0.28            |
| HFS       | Weighted (APO = 8.44 W) | 167              | 1.25            | 13.3            |
|           | Weighted (APO = 21.7 W) | 189              | 1.27            | 11.6            |
|           | Unweighted            | 754.8            | 0.03            | 5.69            |

are summarized for each IFS and HFS exposure condition in Table 4.1.

### 4.3.4 Cavitation simulations

A second series of calculations were conducted to test the assumptions used in the derivation of the correction factor \(\Gamma(f)\). These calculations were designed to demonstrate the feasibility of applying the correction factor to PCD-measured acoustic powers in order to estimate the associated cavitation-generated acoustic power radiated over a given ROI. Additionally, simulation parameters were chosen to contrast the assumptions made in the derivation of the correction factor \(\Gamma(f)\), allowing the model limitations to be explored. This included modeling cavitating bubbles as acoustic sources with constant amplitude and phase to challenge the assumptions of independence and random incoherence as well as positioning bubbles...
with a random distribution to challenge the assumptions of being wide-sense stationarity.

The configurations shown in Figures 4.1(a) and 4.2(a) were again constructed in simulation and the same PCDs, ranges of ROI dimensions and $ka_R$ values used for the calculation of $\Gamma(f)$ in the previous sections were investigated. For the vessel configuration (Figure 4.1(a)), an ensemble of cavitating bubbles was simulated such that a constant number density of 1 bubble per mm$^3$ resided within each volumetric ROI. For the sonophoresis configuration (Figure 4.2(a)), a constant value of 25 cavitating bubbles was simulated within each ROI on the skin surface, providing a number density of 0.08–5 cavitating bubbles per mm$^2$ over the range of investigated ROIs. Cavitating bubbles were simulated at randomized locations with uniform distribution throughout each given ROI for either configuration as coherent monopole radiators.

Cavitation simulations were conducted by employing the Monte Carlo method [Rubinstein and Kroese, 2016]. Specifically, random repeated sampling was conducted over a series of 40 independent cycles by randomizing bubble positions for each observation and calculating the pressure field radiated by the ensemble of cavitating bubbles to represent a time series of cavitation emission measurements for any given configuration, ROI dimension, and frequency. For each cycle, cavitating bubbles were spatially located throughout the given ROI with a random uniform distribution. The Rayleigh integral $P_{\text{Rayl}}$ associated with each PCD and each randomized cavitating bubble was calculated in MATLAB using an exact series expansion method for the focused [Hasegawa et al., 1986] and unfocused [Mast
and Yu, 2005] receivers. Using these values with the associated complex source-strength amplitudes, $Q_i(f)$, PCD surface area, $S_R$, and acoustic impedance ($\rho_0c = 1.49$ MPa·s/m in $25^\circ$ water), the acoustic power, $\Pi_R(f)$, received by the given PCD was calculated following Equation 4.14 for each cycle. For the vessel configuration, the focused and unfocused PCDs were employed to monitor the same cavitating bubbles simultaneously. Concurrently, for either configuration, the corresponding total radiated power, $\Pi_T(f)$, from simulated cavitating bubbles within each ROI was calculated following the analytic expression provided in Equation 4.17. The average of the PCD-measured $\Pi_R(f)$ and total radiated $\Pi_T(f)$ acoustic power values were taken over 40 independent cycles to represent non-deterministic, time-averaged values for each investigated configuration, PCD, frequency $ka_R$, and ROI.

Using the correction factor values, $\Gamma(f)$, calculated in the previous set of calculations for each corresponding ROI, frequency $ka_R$, PCD, and configuration, the simulated time-averaged PCD-measured acoustic powers $\Pi_R(f)$ were corrected following Equation 4.21 where the product of these values was take to estimate the total radiated acoustic power per unit area or volume of the specified ROI. The simulated PCD measurements after correction $\Pi_R(f) \times \Gamma(f)$ were compared with corresponding analytically calculated total radiated power values $\Pi_T(f)/W$ in order to determine the accuracy limits of this method specifically when model assumptions were contrasted. Additionally, for the vessel configuration, the corrected PCD-received powers were compared between the focused and unfocused PCDs to illustrate the system-independent nature of this approach.
4.3.5 Sonophoresis: Correction of PCD-measured cavitation emissions

Subharmonic emission levels associated with stable cavitation activity were shown to be greatly correlated with decreases in skin resistance during IFS and HFS in Chapter 2. However, analyses of emissions were limited to comparison among voltage levels that were measured and influenced by the frequency response of the receiving system. Hence, comparisons among subharmonic emissions measured for intermediate- (IFS) and high-frequency sonophoresis (HFS) were not possible in Chapter 2 due frequency-dependent variations in the response of the system and diffraction effects in the cavitation-radiated field.

In order to compare subharmonic emissions associated with IFS and HFS directly, the associated time-averaged system-measured voltages $X_{sys}(f)$ used in Chapter 2 were first converted to values of the acoustic power $\Pi_R(f)$ received by the PCD. Following Equation 4.29, the acoustic power received by the PCD were calculated using the element surface area $S_R$ of 505.7 mm$^2$, sampling frequency $f_s$ of 10 MHz, period $T$ of each time-domain acquisition of 0.2 s, density $\rho_0$ and sound speed $c$ in room temperature water as 997 kg/m$^3$ and 1491 m/s, respectively, and the calibration factors associated with $M_{sys}(f)$ were those determined in Chapter 3. The frequency bin width $\Delta f$ was 25 kHz after segmenting and averaging each time-domain acquisition with 500 equal length and non-overlapping segments via the method of average periodograms. Each individual segment was windowed with a rectangular window where $w = 1$ and no significant spectral leakage was observed compared to the application of more aggressively tapered functions. Second, using
the frequency-dependent correction factors, $\Gamma(f)$, calculated for the corresponding subharmonic frequencies, for the weighted and unweighted ROIs on the skin surface as shown in Figure 4.7(b) and outlined in Table 4.1, the total power per unit area $\Pi_T(f)/W$ radiated by stable cavitation at the skin surface was estimated following Equation 4.30 at the corresponding subharmonic frequency values where $f = f_0/2$.

4.3.6 Statistical analyses

The relationship between corresponding skin resistance reductions and subharmonic acoustic power radiated by cavitation during IFS and HFS were compared statistically using R software packages [R Core Team, 2013] in the R studio integrated development environment [RStudio Team, 2015]. Correlation coefficients were calculated for data derived from individual sonophoresis trials. Comparisons of correlation coefficients between skin resistance decrease and acoustic power of cavitation radiated per unit area of the skin surface were conducted for dependent data with Williams’ test and for independent data with Fisher’s z-transformation. Linear regression models were also employed to model the data associated with individual trials. Analysis of covariance (ANCOVA) was employed to compare slope and intercept values of corresponding regression models.

Grouped skin resistance decrease and acoustic power of cavitation radiated per unit area of the skin surface data among the various sonophoresis treatments were tested for equality of variance using Levene’s test and tested for normality using Shapiro-Wilk’s test. Grouped data that failed to provide equivalent variance ($p < 0.05$) and normality ($p > 0.05$) were compared using a Kruskal-Wallis rank sum test with corresponding post hoc pair-wise comparisons conducted simultaneously by
adjusting the p-value to account for multiple comparisons using a Holm–Bonferroni method.

Simulated data were analyzed using linear regression to model the relationship between simulated PCD-received powers and analytically calculated acoustic power values, where the simulated PCD-measured values represented the estimator and analytically calculated values represented the known values. Linear hypothesis testing on the regression estimates was conducted to determine significance of one-to-one (i.e., regression slope was equivalent to unity) correspondence between the estimator and known quantities. The mean-squared error between the estimator and known values was calculated to determine the accuracy of the correction factor applied to simulated PCD-received powers.

Analysis was conducted to identify distributional characteristics of stationarity within randomly selected PCD-measured signals obtained during sonophoresis. A total of 13 individual trials were used for further analysis, chosen by random selection such that at least one trial was obtained for each investigated treatment frequency, applied acoustic power, and degassing scheme. First, for each randomly selected trial, 216 individual time traces of 200 ms duration were randomly selected from the 1080 traces acquired per trial. Each trace was segmented by 500 non-overlapping 0.4 ms rectangular windows, the squared magnitude of the discrete Fourier transform of each segment was computed, and the corresponding subharmonic component was extracted as a series of 500 time points over each 200 ms measurement. Second, for each randomly selected trial, the average subharmonic component within each trace was calculated to provide a time series of emissions at 1080 time points over each 30
minute trial. For analysis of either time series, subharmonic emission measurements were normalized to the maximum measured value.

Change point analysis was conducted for each investigated time series measurement to determine when changes in the mean and variance of subharmonic emission levels occurred, where a non-constant mean or variance was used to indicate a lack of stationarity. A Pruned Exact Linear Time (PELT) [Killick et al., 2012] algorithm was employed using R software [R Core Team, 2013] to conduct change point analysis of the randomly sampled time series emission measurements. Algorithm penalty parameters were selected in order to avoid spurious change point detection by first conducting analysis on simulated time series signals with mean and variance changes at known time points. These signals were simulated as Gaussian noise consisting of 400 time points that were normalized to the maximum simulated value in order to correspond in amplitude to the measured emission time series data. Two known change points in simulated data were established by simulating signals with mean and variance values of 0.6 and 0.04, respectively, that were decreased by 20% each at a known time point before returning to approximately the original values at a second known time point. Algorithm penalty values that resulted in proper identification of changes in the mean and variance at known time points in normalized simulated time series signals were 0.5% and 10%, respectively, of the number of time points within the simulated signals. These penalty parameters, employed as 0.5% and 10% of the number of time points within a given time series, were used to find change points in the mean and variance, respectively, of each investigated time series of normalized subharmonic emission measurements.
4.4 Results

4.4.1 Correction factor

For the configuration shown in Figure 4.1(a), representing that of a tube or blood vessel, the correction factor $\Gamma(f)$ was calculated for a focused and unfocused PCD over a range of ROI volumes and frequencies. The calculated correction factor values are shown for all investigated ROI volumes ($2\text{–}105 \text{ mm}^3$) and normalized frequencies ($ka_R = 5\text{–}450$) in Figures 4.5(a) and 4.5(b) for a focused and unfocused PCD, respectively. Notably, for either PCD, the correction factor increases with $ka_R$ approximately as the square of the frequency $k$ and PCD radius $a_R$, and nominally decreases with increasing ROI dimensions.

Although the simulated PCD pair share equivalent radii, the magnitude of the
correction factor for the unfocused PCD for this configuration was typically greater than that of the focused PCD for any given ROI dimensions and frequency, especially at higher $ka_R$ values. To further illustrate this, correction factors for representative frequencies are shown as a function of the ROI volume in Figures 4.6(a) and 4.6(b) for the focused and unfocused PCD, respectively. For the focused PCD, an asterisk indicates the point at which the axial width of the ROI exceeds the -6 dB focal beamwidth of the PCD for each $ka_R$ value. In these plots, the correction factor values are shown to be equivalent between the PCD pair for low frequencies, for example at $ka_R = 10$, but the difference between the correction factor for the focused and unfocused PCD at higher $ka_R$ values increases to several orders of magnitude. This difference is due to the significantly higher sensitivity computed by the Rayleigh
integral associated with the focused PCD compared to the unfocused PCD. For this configuration, the correction for diffraction over the investigated ROI volumes is greater for the unfocused PCD compared to the unfocused PCD, especially at higher $ka_R$ values and even when the ROI width exceeds the -6 dB focal beamwidth of the focused PCD. In addition, these figures show that the correction factor for the focused PCD varies slowly with increasing ROI size, especially for higher $ka_R$ values which provide a narrower axial -6 dB focal beamwidth. The correction factor for the unfocused PCD varies by a value greater than an order of magnitude over the same range of ROI volumes. Hence, one potential approach for focused receivers is to limit the ROI dimensions used for the correction factor calculation to its -6 dB focal beamwidth in order to optimize calculation times. However, for an unfocused PCD, calculation may be required over the entire ROI dimensions due to the broader beam profile of the unfocused receiver.

The correction factor, $\Gamma(f)$, was calculated for a range of skin surface areas and frequencies using the sonophoresis configuration employed in Chapter 2 (Figure 4.2(a)). The calculated correction factor for the sonophoresis configuration is shown in Figure 4.7(a) for ROI areas of 10–350 mm$^2$ and for $ka_R$ values of 5–450. Notably, for any given skin surface area defined as the ROI, the correction factor increased approximately with the square of the normalized frequency $ka_R$, indicating that diffraction effects increase as the square of the frequency $k$ or PCD radius $a_R$ for this configuration. The rapid increase in the correction factor for any $ka_R$ value is due to its inverse relationship with the spatial sensitivity over the ROI, where the spatial sensitivity of the PCD increases near the center of the skin surface plane for
Figure 4.7: (a) The correction factor $\Gamma(f)$ for various circular ROI dimensions on the skin surface as a function of normalized frequency $ka_R$. (b) The normalization for $ka_R$ values of 10.8 (solid) and 50.3 (dashed line), the subharmonic associated with IFS and HFS, respectively, as a function of circular ROI. Values corresponding to normalization factors and ROI dimensions associated with ROIs defined by the Cavitation Index (Δ and □) and over the whole skin surface (○) are marked for each line.

Shown in Figure 4.7(b) are the correction factor values $\Gamma(f)$ as a function of ROI skin surface area calculated specifically for the subharmonic frequencies associated with IFS ($ka_R = 10.8$, solid line) and HFS ($ka_R = 50.3$, dashed line). Additionally, for each frequency the corresponding correction factor $\Gamma(f)$ values and skin surface areas for the various ROI definitions that were based on the unweighted method (circle), or weighted method for the lower (square) and higher (triangle) acoustic powers employed for IFS and HFS are marked. Although the same skin sample
size was used for all sonophoresis trials, the effective ROI dimensions based on the weighted method resulted in smaller characterized skin surface areas for the IFS trials than those of the HFS trials. However, the difference in the correction factor was relatively small between the weighted and unweighted ROIs for IFS and especially for the HFS groups. Similar to previous calculations, the correction factor, $\Gamma(f)$, is shown to increase rapidly with decreasing ROI dimensions when the ROI becomes small, but varies slowly with increasing ROI dimensions as the ROI becomes relatively large. The difference between correction factor values calculated for either frequency at any given ROI size scales approximately with the difference in the square of the normalized frequency $ka_R$.

4.4.2 Simulated emission measurements for a volumetric ROI

Time-averaged values of the simulated PCD-received acoustic power $\Pi_R(f)$ for an unfocused and focused receiver from the same simulated cavitating bubbles within a cylindrical volume are shown on a dB-scale relative to the maximum value measured by either PCD in Figure 4.8(a) as a function of normalized frequency $ka_R$. These values represent quantitative emission measurements which could be made with a calibrated receiver and each plotted point represents the PCD-received power value associated with each individual ROI and normalized frequency $ka_R$ that was investigated. Notably, the received acoustic power values between the unfocused and focused PCD pair, although monitoring the same exact region and cavitating bubbles, provide distinctly different results. Specifically, the focused PCD typically measured significantly greater acoustic powers than that of the unfocused PCD received from the same cavitating bubbles for any frequency and ROI dimensions.
Figure 4.8: (a) Corresponding simulated time-averaged acoustic powers received by an unfocused (●) and focused receiver (×), and the corresponding simulated cumulative cavitation-radiated acoustic powers (∆) as a function of $ka_R$. Each point represents the simulated value for a given region of interest (ROI) and $ka_R$ value. All values are decibel (dB) scaled relative to the maximum simulated value. (b) Corresponding time-averaged acoustic powers $\Pi_R(f)$ simultaneously measured by a focused receiver versus and unfocused receiver after correction by the corresponding $\Gamma(f)$ factor calculated for each normalized frequency $ka_R$ and ROI. A ±1 dB deviation from the solid one-to-one line is indicated by the shaded gray region.

This disagreement in PCD-measurements is a result induced by random phase effects over the different PCD surfaces from the randomized ensemble of cavitating bubbles, which for this configuration had a greater influence over the acoustic power measured by an unfocused receiver in comparison to that measured by a focused receiver. In addition, the measured power values received by either PCD show no inferable correspondence with each other. The corresponding calculated total radiated power per unit volume $\Pi_T(f)/W$ values are also shown for comparison, as dB-scaled values.
normalized to the maximum calculated power. As expected, the ensemble-radiated power increases with frequency and is constant in value for all investigated ROI volumes at any given $k a_R$ value because the number density of cavitating bubbles was held constant for all investigated ROIs. However, the acoustic power values received by the focused or unfocused receivers at any frequency are shown to vary considerably, as diffraction effects vary due to varying ROI dimensions and frequency, and neither show an inferable correspondence with the calculated radiated power values or with the corresponding simulated received value by the other PCD. These results illustrate the insufficiency of quantitative measurements alone, without correction for diffraction, as a standard measurement technique as the acoustic power values received by the two transducers are non-comparable. Furthermore, without correction for diffraction, the acoustic power measured by either transducer was shown to provide a poor estimate of the total acoustic power radiated by cavitation.

Shown in Figure 4.8(b) are simulated PCD-measured acoustic powers made by a focused receiver versus those made simultaneously by an unfocused receiver and normalized to the maximum simulated measurement value after correcting by the corresponding frequency- and PCD-dependent correction factor $\Gamma(f)$ calculated for each ROI volume and normalized frequency $k a_R$. The relationship between corresponding measured values by the different receivers after correction is shown to be linear ($r^2 = 0.94$) with an approximately one-to-one correspondence of the normalized values providing a regression line with a slope not significantly ($p = 0.11$) different from unity. Error was not found to vary with frequency, ROI dimensions, or number of simulated cavitating bubbles. For reference, a $\pm 1 \, \text{dB}$
Figure 4.9: Simulated time-average acoustic powers $\Pi_R$ measured by a (a) focused ($\times$) and (b) unfocused ($\circ$) PCD after correction by the corresponding $\Gamma$ factor calculated for each normalized frequency $ka_R$ and region of interest (ROI) versus the corresponding total radiated power values over a given ROI. A ±1 dB deviation from the one-to-one line is indicated by the shaded gray region.

Figures 4.9(a) and 4.9(b) show the simulated time-average acoustic powers, $\Pi_R(f)$, measured by a focused and unfocused PCD, respectively, after correction for
diffraction with the factor $\Gamma(f)$ for each corresponding ROI volume and $ka_R$ value to provide an estimate of the total radiated power per unit volume, $\Pi_T(f)/W$. These estimated values are plotted against corresponding total radiated power per unit volume values calculated analytically, where each plotted point represents the value corresponding to each investigated ROI and $ka_R$ value. All values are normalized to the maximum calculated $\Pi_T(f)/W$ value and the shaded gray region in each plot indicates a deviation from the one-to-one line of $\pm 1 \text{ dB}$.

Notably, after correction of the focused PCD-measured acoustic power (Figure 4.9(a)), the correspondence between the estimated power from simulated measurements and calculated power was found to be linear ($r^2 = 0.96$). Likewise, for the unfocused PCD measurements after correction (Figure 4.9(b)), the correspondence between the estimated power from simulated measurements and calculated power was also found to be linear ($r^2 = 0.98$). Furthermore, the linear relationships between the corrected focused and unfocused PCD measurements with the calculated power was found to have a slope not significantly ($p = 0.12$ and $p = 0.17$, respectively) different from unity, indicating a strong one-to-one correspondence.

The results shown in Figures 4.9(a) and 4.9(b) for the focused and unfocused PCD, respectively, indicate that the PCD received acoustic power from simulated cavitating bubbles accumulated over the PCD surface coherently on average. Hence, the agreement between the corrected focused and unfocused simulated measurements agreed with the calculated values with relatively low mean squared error (MSE) of $3.33 \times 10^{-3}$ and $1.55 \times 10^{-3}$, respectively. Deviations from the one-to-one linear
correspondence were likely due to imposed simulation limitations that contrasted model assumptions in the calculation of the correction factor, such as the uniform amplitude and phase as well as the sparse density of cavitating bubbles within the associated ROI used for simulations. In addition to providing accurate estimates of the calculated value, the corrected PCD-measured values corresponded between different receivers (Figure 4.8(b)).

4.4.3 Cavitation simulations for a surface ROI

Time-averaged, uncorrected values of the calculated total-radiated acoustic power $\Pi_T(f)$ and simulated PCD-received $\Pi_R(f)$ acoustic power, normalized to the respective maximum values, are shown in Fig. 4.10(a) as a function of normalized frequency $ka_R$. Each plotted point represents the PCD-received power value for each ROI and normalized frequencies $ka_R$. Time-averaged total radiated power $\Pi_T(f)$ values are shown to increase approximately as the square of $ka_R$, independently of the ROI due to a constant number of simulated cavitating bubbles for each condition. However, time-averages of the PCD-received powers $\Pi_R(f)$ are shown to vary significantly and unpredictably with $ka_R$ and the ROI over which they were calculated, a result induced by random phase effects over the PCD surface from the randomized ensemble of cavitating bubbles. Although shown as normalized values, this figure illustrates the lack of any inferable one-to-one correspondence between corresponding time-averaged values of $\Pi_T(f)$ and $\Pi_R(f)$, further illustrating the insufficiency of quantitative measurements alone as a standard measurement technique.

In Fig. 4.10(b) normalized time-averaged values of the total radiated power per
Figure 4.10: (a) Uncorrected, time-averaged PCD-received (dots) and total radiated (triangles) acoustic powers as a function of $ka_R$. Each point represents the time-averaged value calculated for a given ROI and $ka_R$ value. (b) Corrected PCD-received acoustic power versus corresponding total radiated power values over a given ROI. A $\pm 1$ dB deviation from the solid one-to-one line is indicated by the shaded gray region.

Unit area $\Pi_T(f)/W$ are plotted against simulated acoustic powers measured by the PCD $\Pi_R(f)$ after correction by the calculated factor $\Gamma(f)$. Each plotted point represents the corresponding simulated and calculated acoustic power value for each investigated ROI and $ka_R$. Despite employing simulation conditions that severely contrasted model assumptions, agreement between these corresponding values is shown to be approximately within $\pm 1$ dB, indicated by the shaded region extending above and below the one-to-one line, for all investigated $ka_R$ and ROI values. Linear regression applied to the relationship between the simulated PCD-received powers after correction and the calculated total radiated acoustic power per unit area was
linear \( r^2 = 0.986 \) with relatively low MSE \( (2.5 \times 10^{-4}) \). Deviations from the one-one linear correspondence are likely due to imposed simulation limitations, such as the uniform phase and source-strength amplitude of cavitating bubbles as well as the sparse density of bubbles within each ROI. This agreement further indicates the element-received power from multiple cavitating bubbles combine approximately incoherently.

### 4.4.4 Sonophoresis experiments: Quantitative spectral analysis

Shown in Figure 4.11 are representative spectra of the measured voltage (a, d), the average acoustic power over the PCD surface (b, e), and the cavitation-radiated acoustic power per unit area of the skin surface (c, f) during IFS and HFS, respectively. For each plot, black lines indicate a trial where little or no cavitation was detected, and red and blue lines are representative trials when different levels of emissions from cavitation were monitored. The subharmonic components associated with emissions from stable cavitation are highlighted in each figure.

Figures 4.11(a) and 4.11(d) show the system-measured voltages prior to correction are shown for representative IFS and HFS trials, respectively. The spectra indicated by black lines were from control trials in which little or no emissions from cavitation were detected, hence these lines represent the respective electronic noise floor. The bandwidth for monitoring emissions from cavitation during IFS is shown to be especially narrow due to filtering that reduced the usable signal for analysis over a frequency range of approximately 0.1–0.25 MHz, making it challenging to interpret broadband signal characteristics for these trials. On the other
Figure 4.11: Representative spectra as a function of frequency of the system-measured voltage (a,d), average incident acoustic power on the PCD (b,e), and the estimated acoustic power per unit area (c,f) obtained during IFS and HFS, respectively. Black dashed lines in each represent a trial that little to no emissions from cavitation were measured and represent the approximate noise floor. Subharmonic $f_0/2$ components are highlighted for IFS ($f_0/2 = 0.205$ MHz) and HFS ($f_0/2 = 1.0$ MHz) trials.

In contrast, a broader usable bandwidth was obtained for HFS trials as shown in Figure 4.11(d) where the signal from cavitation emissions remained distinguishable from the
electronic noise floor from approximately 0.2–1.5 MHz. However, over the respective usable bandwidth for each treatment regime, the broadband behavior appears similar between approximately the 1/3 and 1/2 harmonics of the corresponding insonation frequencies.

Figures 4.11(b) and 4.11(e) show the representative spectra of the acoustic power received by the PCD surface from cavitation during IFS and HFS. These spectra were calculated as the PDS outlined in Equation 4.29. Additionally, these spectra correspond to the voltage measurements in Figures 4.11(a) and 4.11(d) after correction for the frequency response and sensitivity of the PCD and corresponding electronics using values that were shown in Figure 3.16(b). For the trials which resulted in little or no cavitation activity, although scaled by the correction factor \( \Gamma(f) \), the sensitivity had little to no effect on these measured values and the spectra indicate the minimum sensitivity to the received acoustic powers. After removal of the influence imposed on the voltage measurements by the receiving system, the acoustic power received by the PCD during the HFS trials in which cavitation emissions were monitored are shown to result in nearly constant acoustic power with respect to frequency over the PCD surface for non-peak frequency components within the signal bandwidth of 0.2–1.5 MHz. This relatively constant increase above the noise floor for these trials is presumably due to measured broadband emissions from inertial cavitation.

Finally, after applying the unweighted correction factor \( \Gamma(f) \) using the values shown in Figure 4.7 to the PCD-received acoustic powers, the corresponding spectra for the cavitation-radiated acoustic power per unit area of the skin surface
were calculated and are shown in Figures 4.11(c) and 4.11(f) for IFS and HFS, respectively. Notably, the contents of these spectra can now be compared directly between IFS and HFS due to correction for diffraction effects and the frequency response of the system, where these spectra directly characterize the cavitation-radiated powers on the skin surface. For HFS trials in which cavitation emissions were monitored, over a bandwidth of 0.2–1.5 MHz before the signal-to-noise is significantly diminished, the spectra increase approximately as the frequency squared, which is consistent with the analytic expression for the average cavitation-radiated power shown in Equation 4.3. Following these representative plots, the subharmonic component was extracted from each individual sonophoresis trial and used for further analysis in the following sections.

Change point analysis was conducted on subharmonic time series measurements consisting of over 2,800 individual traces, each consisting of 500 time points over a duration of 200 ms, and 13 individual trials, each consisting of 1,080 time points over a duration of 30 minutes. Approximately 83% and 7% of the randomly selected 30 minute trials were found to provide no change point in the mean and variance, respectively, of the squared-voltage associated with subharmonic emissions over the duration of treatment. Similarly, approximately 97% and 85% of the randomly selected 200 ms duration traces were found to provide no change point in the mean and variance, respectively, of the squared-voltage associated with subharmonic emissions. These results indicate that the squared-voltage subharmonic levels over the duration of each measurement were mean-stationary, but do not indicate stationarity of the variance, especially over the 30 minute duration of trials. However,
the derivation of the diffraction correction method only requires stationarity of the mean in the measured squared voltage amplitude, proportional to the acoustic power, over the duration of the measurement used to estimate average power spectra. For the measurements and analysis conducted for sonophoresis, average power spectra were estimated from individual time-domain traces, which were found to be mean-stationary. These results indicate consistency with the assumptions made in the derivation of the diffraction correction factor and justify its application to emission measurements made during sonophoresis.

4.4.5 Sonophoresis experiments: Characterization of cavitation-radiated subharmonic acoustic powers over an unweighted region of interest

The PCD-measured subharmonic acoustic powers monitored during sonophoresis were corrected by the frequency-dependent correction factor, $\Gamma(f)$, (shown in Figure 4.7(b)) calculated using an unweighted ROI in order to characterize the acoustic power radiated by cavitation per unit area of the treated skin surface for each individual trial. For this ROI definition, radiated powers from cavitating bubbles were characterized over a skin surface area of $754.8 \text{ mm}^2$ for all treatments, independent of the insonation frequency or power. Shown in Figure 4.12(a) are the mean values of the dB-scaled radiated subharmonic acoustic powers per unit area of the skin for each treatment group against corresponding mean values of skin resistance decrease. Vertical and horizontal lines indicate one standard deviation associated with each group for skin resistance and normalized acoustic power, respectively. For these values, two distinguishable groups of data were identified:
Figure 4.12: Normalized skin resistance decreases and decibel-scaled acoustic powers normalized using an unweighted correction factor calculated over the whole skin surface. (a) Mean and standard deviation of the normalized skin resistance decreases and decibel-scaled acoustic powers for each treatment group. (b) Skin resistance decrease values as a function of corresponding decibel-scaled acoustic powers for each individual IFS (red) and HFS (blue) trial. The dashed line indicates the linear least squares regression line applied to all data points.

(1) treatments that resulted in low radiated subharmonic acoustic powers and low skin resistance reductions and (2) treatments that resulted in comparatively higher radiated subharmonic acoustic powers and higher skin resistance reductions. Comparisons among groups that produced minimal subharmonic acoustic powers and skin resistance reductions revealed that there was no significant difference ($p > 0.1$) in either value between IFS-treated PBS-degassed (dg), HFS- and IFS-treated control trials. Likewise, comparisons among groups that produced comparatively
higher subharmonic acoustic powers and skin resistance reductions revealed that there was no significant difference \((p > 0.05)\) in either value between HFS-treated PBS-degassed, IFS- and HFS-treated skin-degassed trials. Additionally, the latter treatment groups resulted in significantly \((p < 0.05)\) greater normalized subharmonic powers and significantly \((p < 0.03)\) greater skin resistance reductions than the former treatment groups. These results are not dissimilar to those determined in Chapter 2, as increases in subharmonic emissions were found to be accompanied by greater skin resistance decreases. However, correcting measured emissions to estimate the cavitation radiated acoustic power on the skin surface provides IFS- and HFS-measured emissions that are now on an equivalent scale, illustrating a correspondence between radiated subharmonic powers and skin resistance decreases for IFS and HFS, independent of the treatment frequency.

Shown in Figure 4.12(b) are decibel-scaled (dB-scaled) radiated power estimates using an unweighted correction factor, \(\Gamma(f)\), against corresponding normalized skin resistance reduction values measured for each individual IFS and HFS trial. Similar to the results of Chapter 2, significant \((p < 10^{-10})\) log-linear correlations were found for both IFS \((r = 0.94)\) and HFS \((r = 0.859)\) radiated subharmonic powers against corresponding skin resistance reductions. Although the independently calculated correlation coefficient for IFS trials was greater than that of HFS trials, the difference was not significant \((p = 0.1)\). With the measurements now scaled to equivalent values, the correlation coefficient of the IFS and HFS trials combined was calculated to determine the if a linear relationship exists between skin resistance decreases and subharmonic acoustic powers independently of the applied treatment frequency.
Combining the IFS and HFS values resulted in a significant \( p < 10^{-10} \) correlation coefficient of \( r = 0.820 \) between the radiated acoustic power values and skin resistance decreases, which was not found to be significantly different \( p > 0.08 \) from that of the IFS or HFS results alone. The linear least squares regression line associated with the relationship between radiated powers on the skin surface and changes in skin resistance is shown in 4.12(b) as a dashed line.

### 4.4.6 Sonophoresis measurements: Characterization of cavitation-radiated subharmonic acoustic powers over a weighted region of interest

The PCD-measured subharmonic acoustic powers monitored during sonophoresis were corrected by the frequency-dependent correction factor \( \Gamma(f) \) (shown in Figure 4.7(b)) calculated using a weighted ROI in order to characterize the cavitation power radiated per unit area of the treated skin surface. In comparison to the previous results, here the ROI was weighted using the Cavitation Index and transmit beam, resulting in varying skin surface areas that were characterized for each treatment frequency and applied acoustic power as summarized in Table 4.1. The characterized skin surface areas for IFS were 30.3 and 100.3 mm\(^2\) for treatment powers of 0.79 and 1.68 W, approximately 4% and 13% of the total skin surface area, respectively. For HFS-treated skin, the characterized skin surface areas for were 167 and 189 mm\(^2\) for treatment powers of 8.44 and 21.7 W, approximately 22% and 25% of the total skin surface area and 550% and 188% larger than corresponding IFS-characterized skin surface areas, respectively.

Shown in Figure 4.13(a) are the mean values of the dB-scaled radiated
subharmonic acoustic powers per unit area of the skin for each treatment group against corresponding mean values of skin resistance decrease. Vertical and horizontal lines indicate one standard deviation associated with each group for skin resistance and normalized acoustic power, respectively. Similar to the comparisons made for cavitation radiated powers characterized from the full skin surface, no significant difference ($p > 0.1$) in either value between IFS-treated PBS-degassed (dg), HFS- and IFS-treated control trials was found. Likewise, despite the different ROI dimensions for each treatment group, no significant difference ($p > 0.05$) in either value among HFS-treated PBS-degassed, IFS- and HFS-treated skin-degassed trials was found. Additionally, the latter treatment groups resulted in significantly ($p < 0.05$) greater normalized subharmonic powers and significantly ($p < 0.03$) greater skin resistance reductions than the former treatment groups.

Shown in Figure 4.13(b) are decibel-scaled (dB-scaled) radiated power estimates using a weighted correction factor $\Gamma(f)$ against corresponding normalized skin resistance reduction values measured for each individual IFS and HFS trial. Significant ($p < 10^{-10}$) log-linear correlations were found for both IFS ($r = 0.925$) and HFS ($r = 0.858$) corrected subharmonic acoustic powers against corresponding skin resistance reductions. Although the independently calculated correlation coefficient for IFS trials was greater than that of HFS trials, the difference was not significant ($p = 0.21$). Combining the values for all IFS and HFS trials resulted in a significant ($p < 10^{-10}$) correlation with a coefficient of $r = 0.823$, which was not found to be significantly different ($p > 0.07$) from that of the IFS or HFS results alone. The linear least squares regression line associated with this
Figure 4.13: Normalized skin resistance decreases and decibel-scaled acoustic powers normalized using a correction factor weighted by the Cavitation Index and transmit beam for each treatment group. (a) Mean and standard deviation of the normalized skin resistances decrease and decibel-scaled acoustic powers for each treatment group. (b) Skin resistance decrease values as a function of corresponding decibel-scaled acoustic powers for each individual IFS (red) and HFS (blue) trial. The dashed line indicates the linear least squares regression line applied to all data points.

The dashed lines in Figures 4.12(b) and 4.13(b) indicate the regression line used to model all subharmonic emission levels with associated reductions in skin resistance values normalized using a weighted and unweighted ROI, respectively. The parameters of the regression lines associated with each method were compared using analysis of covariance (ANCOVA), revealing no significant difference between
the slope ($p > 0.83$) or intercept ($p > 0.06$) values when the radiated subharmonic power was normalized using a weighted or unweighted correction factor resulting in different scaling factors. This indicates that although different skin surface areas were characterized, both ROI definitions resulted in equivalent relationships between normalized subharmonic emissions during IFS and HFS and resultant decreases in skin resistance for these experiments.

### 4.5 Discussion and conclusions

The work presented here provides a method for normalizing PCD-received emissions for quantitative analysis independent of the employed experimental configurations, PCD, or frequency of interest. First, this is accomplished by calibrating the receive system in order to determine the cavitation-radiated pressures, and by extension the power, received by a PCD. Second, a correction factor was derived (Equation 4.20) relating the PCD-received power to that radiated by cavitation throughout a defined region of interest (ROI) by accounting for diffraction effects. This derived factor is provided as a simple analytic expression that can be obtained computationally, requiring only the calculation of the Rayleigh integral over a discrete uniform grid throughout a defined ROI in order to account for the relative spatial sensitivity of the PCD to cavitating bubbles within its field-of-view. The feasibility of this application was demonstrated in simulation and representatively applied to emission measurements made during sonophoresis experiments.

In past studies, the system-measured voltage generated upon a PCD receiving cavitation-radiated emissions has often been used to predict bioeffects such as
cellular damage [Hallow et al., 2006, Chen et al., 2003c, Hwang et al., 2006] and vessel rupture [Hoerig et al., 2014], or provide an estimate of the probability of cavitation activity onset [Khokhlova et al., 2009, Maxwell et al., 2010, Li et al., 2014]. Although providing useful information, one limitation of these studies is that the results are dependent on and specific to the employed receiving system and experimental configuration. Using the system generated voltage for analysis provides a significant challenge in predicting or estimating a given bioeffect from cavitation emissions when these components cannot be identically replicated. Hence, for a given experiment type, standard experiments may need to be replicated in order to reestablish the relationship between cavitation emission levels and the onset of a given bioeffect whenever experimental or receive components are changed. This challenge was presented in the sonophoresis trials explored here. Although the same configuration was employed for IFS and HFS, the frequencies of interest associated with each treatment were different. Because the receive sensitivity and diffraction effects that influenced emission measurements for IFS and HFS were different, the system- or PCD-generated voltages acquired during cavitation detection were not comparable.

Absolute pressure measurements are one alternative that provide useful information about cavitation by eliminating the frequency response of the receiving system and providing measurements in proper physical units. For example, an exact characterization of the pressure or power radiated by cavitation can be conducted via absolute pressure measurements for single bubble experiments if the cavitating bubble location is precisely known and is at the geometric focus of the PCD receiver.
[Collin and Coussios, 2011]. For this specific case, absolute pressure measurements by the receiver are sufficient as a system-independent, standard measurement because diffraction effects do not influence the pressure received by the PCD.

However, for the case in which cavitating bubbles are distributed at multiple unknown locations, absolute pressure or power measurements over the element surface are not sufficient as a standard measurement technique because the PCD geometry, position relative to cavitating bubbles, and frequency of emissions provide varying diffraction effects that influence the measured pressure field over the element surface. The potential for disagreement between measurements made using different receiving configurations was shown via numerical results here (Figures 4.8(a) and 4.10(a)) as the receiver-measured acoustic powers varied unpredictably as the frequency, ROI dimensions, and PCD geometry were varied. In addition, the PCD-received powers were shown to vary drastically between the different receivers despite motoring the same cavitation events, and these powers were shown to be poor predictors of the true cavitation-radiated powers. However, after correction of the simulated PCD-received powers by the derived factor $\Gamma(f)$, the simulated power measurements were shown as relatively accurate estimates of the true, total radiated powers per unit area or volume of the ROI (Figures 4.9 and 4.10(b)). Equally as important, measurements made by different receivers of the same cavitating bubbles were also shown to be equivalent after correction (Figure Figures 4.8(b)), independent of the PCD geometry, ROI dimensions, or frequency of emissions.

Although the derived methods here allow cavitation activity to be directly characterized from PCD-measured emissions, there are limitations to the accuracy of
this approach that are primarily due to the inability to spatially resolve the precise locations of cavitation, that may result from and lead to an erroneous definition of the region of interest. First, by defining the ROI margins well beyond the true boundaries of cavitation activity, the power per unit area or volume estimation will be reduced. This alone may not provide inaccuracy, depending on the area or volume being characterized, as the spatial spread of radiated power will naturally decrease with increasing ROI dimensions. Hence, a conservative estimate of the ROI dimensions will provide characterization of a larger region leading to a reduced power per unit area or volume. However, incorporating a correction for diffraction from locations not occupied by cavitating bubbles may induce error in the conversion from element-received powers to the characterized power per unit area or volume.

Error induced by incorporating spatial locations not occupied by a cavitating bubble in the calculation of the correction factor was in part considered in the cavitation simulations here as the number density of cavitating bubbles was held to relatively low values and were simulated with random uniform distribution instead of a discrete uniform distribution over which the correction factor was calculated. Hence, the correction factor was calculated over multiple locations that were not occupied by cavitating bubbles during cavitation simulations. Yet the disagreement between the conversion of element-received powers and the true total cavitation-radiated powers was shown to typically not exceed ± 1 dB. The observed error is presumably an upper limit for the investigated configurations since the number density of cavitating bubbles and number of independent observations used for averaging were both maintained to relatively low values in simulation.
For experimental measurements, this error may be reduced due to an increase in the number density of cavitating bubbles and number of observations to average measured values over.

Second, underestimating the boundaries of the region containing cavitating bubbles, such that activity occurs outside the ROI, may provide a greater source of error especially if the radiated emissions significantly influence the receiver-measured signal. A conservative estimate of the ROI margins may be less prone to this type of error at the cost of increasing the potential for error described previously by overestimating the boundaries of the ROI. For example, cavitation activity induced during sonophoresis could occur within the bulk medium coupling the treatment transducer to the skin, at locations not accounted for even with a conservative ROI definition since only the skin surface was characterized. For the trials investigated here, this was presumed to be unlikely since measured emission levels obtained during HFS trials were comparable when cavitation was isolated to the bulk medium or to within the skin only, indicating that the majority of cavitation occurred near the skin surface. Additionally, due to the smaller skin surface areas associated with the weighted ROI in comparison to the conservative unweighted ROI, cavitation could additionally occur at the skin surface outside of the defined ROI. However, the probability of consistent cavitation activity occurring outside this ROI was assumed to be low due to the rapid decrease in the transmit pressure on the skin surface in the azimuth direction for all treatment regimes (Figures 4.3(a) and 4.4(b)).

Third, in addition to error provided by the ROI estimation and associated correction factor calculation, uncertainty in the PCD receive sensitivity calibration

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may provide error that is inherent to absolute pressure measurements leading to error in the estimated cavitation-radiated power. The majority of the frequency-dependent calibration uncertainty was shown here to arise from the uncertainty of the manufacturer-calibrated hydrophone sensitivity and is therefore unavoidable. For the PCD calibrated in Chapter 3, the total receive sensitivity uncertainty at 0.205 and 1 MHz, the subharmonic frequencies of interest for IFS and HFS, respectively, was 39.68% and 19.42%. This calibration uncertainty corresponds to approximately a ±3 and ±2 dB uncertainty in the PCD-measured acoustic power at 0.205 and 1 MHz, respectively.

Characterizing cavitation on the skin surface for the sonophoresis trials investigated here using a weighted ROI resulted in a skin surface areas that were 4–13% and 22–25% for IFS and HFS (Table 4.1), respectively, of the total skin surface area defined by an unweighted ROI. Despite these considerably smaller skin surface areas and corresponding correction factors used to normalize measured emissions, the relationships between dB-scaled cavitation-radiated power per unit area and changes in skin resistance were shown to be comparable and independent of the ROI definition (Figures 4.12 and 4.13). For either ROI definition and insonation frequency, the onset of significant changes of skin resistance (10% reduction or greater) occurred when the time-average power per unit area of the skin surface exceeded approximately 1 \( \mu \text{W/mm}^2 \). This approximate threshold value varies only by 0.5–2 \( \mu \text{W/mm}^2 \) when considering the uncertainty provided by the calibration of the PCD of up to ±3 dB in power. Because this relationship was shown to be linear on a log-linear scale, the uncertainty contributed from the PCD calibration
or variations in the ROI characterization were marginal.

The corrected emission measurements made for sonophoresis were used as an example to compare results obtained using different PCD measurement systems and frequencies. In the context of sonophoresis, previous studies have speculated that the use of lower frequency ultrasound for sonophoresis may be superior to the use of higher frequency ultrasound due to an increased occurrence of larger cavitating bubbles at lower frequencies [Polat et al., 2011]. However, the results shown here illustrate that the resultant increase in skin permeability incurred during sonophoresis is directly related to the cavitation-radiated acoustic power and is independent of the insonation frequency. Hence, one approach to optimizing sonophoresis treatments is to provide a greater number of cavitation nuclei via the use of ultrasound contrast agents (UCA) [Park et al., 2010, Park et al., 2012]. Because UCAs are manufactured within a size distribution resonant to high-frequency ultrasound (approximately 2–8 MHz) [Klibanov, 2002], HFS treatments may be optimized to improve efficiency and potency with the inclusion of UCAs in comparison to lower frequency applications. In addition, this approach may enable the use of lower insonation pressures at higher frequencies, therefore reducing the total acoustic energy that skin would need to be exposed to for a given treatment as well as reducing the risk of tangential occurrences of cavitation outside of the ROI or away from contrast agents, as well as bioeffects associated with heating and radiation forces.

Theory and feasibility of normalizing emissions measurements to characterize the power radiated by cavitation over a region of interest were shown here. In
addition, an example analysis of normalized emissions measured during sonophoresis was conducted using two example ROI definitions. Although limitations in accuracy are known to exist, these errors were shown via simulation to be comparable to the uncertainty contributed through transducer calibration. For the example application explored here, the acoustic power per unit area of the skin surface in broadband emissions from inertial cavitation were not quantified because subharmonic emissions more consistently predicted the presence or absence of the sonophoresis effect. Hence, future research should be conducted to experimentally validate these methods as well as include analysis of broadband emissions.

Although the approach derived for characterization of cavitation radiated acoustic powers in this chapter is theoretically derived for any PCD and configuration, steps may be taken for practical optimization. In particular, equally important and complementary aspects can be elucidated for the design of the PCD system as well as for the exposure conditions employed for cavitation-mediated therapies. Although it is already common practice to characterize the transmit field of a given treatment transducer, additional consideration may be given to the beam pattern characteristics within a target ROI when conducting treatment planning. First, treatment planning may include identification of a source transducer that is capable of providing a pressure profile that sufficiently encompasses and is relatively uniform throughout the target volume or surface. Second, a treatment transducer may be selected such that it is capable of providing an insonation pressure that sufficiently exceeds the threshold to initiate cavitation throughout the target volume or surface. These steps may be taken to provide cavitation activity with
greater likelihood of occurrence as well as with greater spatial consistency over the target volume or surface because the probability, persistence, and threshold to initiate cavitation activity is in part dependent on the amplitude of the insonation pressure [Khokhlova et al., 2009, Maxwell et al., 2013, Li et al., 2014]. Hence, a beam profile that varies spatially over a given ROI such that the amplitude varies above and below the threshold to initiate cavitation would not be desirable as the probability distribution of cavitation throughout the ROI would be non-constant. In comparison, the pressure profiles generated over the skin surface during sonophoresis, shown in Figures 4.3(a) and 4.4(a) for IFS and HFS, were generally uniform as they each provided a consistent pressure amplitude that exceeded the calculated pressure associated with the Cavitation Index for resonant sized bubbles.

For design of a PCD configuration, many of the apparent characteristics of a receiver should be considered. These considerations include the frequency bandwidth of the PCD, such that it provides adequate SNR for the frequencies of interest. However, in order to improve the sensitivity and accuracy of quantitative emission measurements, additional consideration should be given to the PCD and its alignment with a given ROI. Specifically, the geometry of the spatial sensitivity pattern provided by the PCD would ideally match the geometry of the ROI, coinciding with the geometry of the transmit pressure throughout the region, and provide uniform coverage. For geometrically focused PCDs, alignment is straightforward as the focal region of the PCD can be placed within the ROI for all frequencies. In order to obtain uniform coverage within the ROI, an ideal focused PCD would be selected such that its lateral and on axis -6 dB beamwidth
is sufficiently wide to encompass the defined ROI at the minimum frequency of interest. For example, the simulated spatial sensitivity of a focused PCD in Figure 4.1(b) illustrates an example where the focal region is placed within the ROI, with a spatial varying sensitivity pattern that consists of a -6 dB beamwidth that exceeds the on axis width of the example ROI. However, for this PCD and frequency example, the lateral sensitivity varies such that highly nonuniform coverage is provided throughout the ROI in the lateral direction, with multiple nulls that induce areas of low sensitivity to cavitation within the ROI.

In comparison to focused PCDs, for a given unfocused PCD, focal alignment becomes complicated by the fact that the focal region is not geometrically fixed in space for all frequencies, varying in axial distance from the PCD due to its frequency-dependent diffraction pattern as $z_{\text{lam}} = ka_R^2/2\pi$. For use of an unfocused PCD, if the frequency of interest comprises of a single value, alignment can be conducted in the same manner as for a focused transducer by placing the focal region of the PCD at the frequency of interest within the ROI. However, many applications require simultaneous sensitivity to multiple frequencies within the same ROI. For these applications, in order to provide uniform coverage throughout a given ROI, the PCD should be placed at a distance such that the focal region of the lowest frequency of interest resides within the ROI such that the sensitivity of the PCD for higher frequency components resides within the near field, where its spatial sensitivity is more slowly varying. Further, in order to prevent large variations in the spatial sensitivity in the lateral direction, the diameter of the unfocused PCD should be sufficiently larger than the diameter of the ROI being interrogated. For example,
in Figure 4.1(c) the near field diffraction pattern of an unfocused PCD within the example ROI is shown, where the diameter of the PCD is approximately twice as large as the diameter of the example ROI. For this example, relatively uniform spatial sensitivity to cavitation activity throughout the ROI is obtained, where there spatial sensitivity varies only by approximately 12 dB throughout the ROI. For comparison, the sensitivity of the example focused PCD (4.1(b)) to cavitation within same ROI for the same frequency varies over a range of 40 dB. Hence, the geometry of the target ROI should be considered when selecting a treatment transducer as well as the type and size of PCD to employ. For optimization, selection of a PCD for a given ROI can be evaluated by conducting simulations to determine the uniformity of sensitivity for the frequencies of interest.
Chapter 5

Chapter 5: Conclusions and Future Directions

5.1 Summary and Conclusions

The aim of the work described in this dissertation was to characterize acoustic cavitation and associated bioeffects during therapeutic ultrasound. Specifically, this included identifying and distinguishing the role of cavitation in the enhancement of skin permeability during intermediate- (IFS) and high-frequency sonophoresis (HFS) using quantitative, system-independent measurements of radiated acoustic emissions in order to characterize cavitation.

In Chapter 2, cavitation was confirmed to be the primary enhancement mechanism of IFS and HFS via a series of in vitro experiments. This was accomplished by assessing cavitation activity using passive cavitation detection techniques to monitor radiated emissions. Analysis of these measurements showed that significant skin resistance decreases, indicating increases in permeability, occurred only in the presence of subharmonic emissions associated with stable

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cavitation. In Chapter 3, techniques were developed to accurately characterize the frequency-dependent receive sensitivity of single-element receivers in order to make absolute measurements of the cavitation-radiated pressure incident on a passive cavitation detector (PCD). In Chapter 4, a quantitative and system-independent measurement and analysis technique was developed for characterization of the acoustic power radiated by cavitation within a defined region of interest (ROI). Using the methods developed in Chapter 3 and Chapter 4, quantitative analysis was conducted on the PCD measurements made during sonophoresis in Chapter 2 in order to physically characterize the radiated acoustic power from cavitation over the skin surface during sonophoresis. In addition to discovering the mechanisms by which skin is permeabilized during sonophoresis, this dissertation provides cavitation emission measurement and analysis methods that may be employed as standard techniques in various other therapeutic applications to characterize cavitation-radiated powers.

In Chapter 2, the dependence of skin permeability enhancement during IFS and HFS on acoustic cavitation was demonstrated. The specific location(s) and type(s) of cavitation that lead to enhanced skin permeability were shown to be different between the two frequency regimes. When skin was treated with HFS, significant acoustic emissions from cavitation were accompanied with significant reductions of skin resistance when cavitation was isolated either outside of the skin only or within the skin only. The initial rapid decrease and the end-of-treatment reduction of skin resistance during HFS was shown to correlate significantly greater with subharmonic than with broadband emissions, emanating from stable
and inertial cavitation, respectively. When skin was treated with IFS, significant acoustic emissions from cavitation and significant reductions of skin resistance were observed exclusively when cavitation was isolated outside of the skin only. The initial rapid decrease of skin resistance during IFS correlated equally with subharmonic and broadband emissions, while the total relative reduction was shown to correlate significantly greater with subharmonic emissions.

The reduction of skin resistance incurred during treatment using either investigated frequency regime was shown to be reversible after all treatments. However, skin resistance recovered more slowly and required a greater time duration to fully recover after HFS than after IFS treatment. The results of this study may be used in future investigations to improve IFS and HFS efficacy by designing treatments to exploit the specific cavitation mechanisms associated with each frequency regime identified here in order to reduce required treatment times while also maximizing skin permeabilization. Furthermore, the frequency regime used for treatment can be chosen considering the time required for the skin barrier to recover.

In Chapter 3, two methods for calibrating the absolute receive sensitivity of single-element transducers were developed. These methods, in addition to a third method referred to as a bistatic scattering substitution method [Collin and Coussios, 2011], were investigated numerically to determine measurement configurations that provide accurate calibration measurements of focused receivers. Specifically, calibration accuracy was investigated by comparing simulated reference pressure measurements to corresponding simulated receiver-measured pressures.
Each investigated method was shown to be accurate under given conditions, and can be conducted without using a correction factor for diffraction as is typically needed for calibrations conducted using reciprocity techniques [Zhang et al., 2016].

For a bistatic scattering substitution method, the sound wave scattered by a spherical scatterer is statically measured with a calibrated hydrophone and used as the reference measurement. The accuracy between this measurement and that which is measured by the uncalibrated receiver, and by extension the calibration accuracy, is dependent on the spherical directivity of the scattered wave relative to the curved surface of a focused receiver. The agreement between these measurements was assessed and the accuracy of this method was shown to be sensitive to the calibration frequency, scatterer size and material, and the geometry of the transducer to be calibrated. For example, in order to maintain a maximum of ±1 dB in measurement error when using a silica scatterer, the scatterer diameter must be less than 1/2 of the −6 dB focal beamwidth of the focused receiver at the greatest frequency of interest.

In comparison to a bistatic scattering substitution method, the reference pressure used in a pulse-echo and pitch-catch technique, both developed in Chapter 3, is acquired by using an approximation calculated from planar hydrophone measurements to represent the receiver-measured pressure. The accuracy of this approach was assessed for focused receivers and the accuracy of a pitch-catch technique was found to be dependent on the geometry of the focused source relative to that of the focused receiver to be calibrated. Specifically, to maintain relatively low measurement error (≤±1 dB) using the pitch-catch method, a focused source
with an equivalent or smaller \( f \)-number than that of the receiver is required. In comparison, low calibration measurement error (\( \leq \pm 1 \) dB) using the pulse-echo technique was found for all investigated frequencies and receiver geometries. These parameters should be considered when determining the components used in future calibrations of focused transducers to be used as passive cavitation detectors to ensure acceptable levels of calibration error are maintained.

Although a bistatic scattering substitution method is applicable only for focused receivers, the pulse-echo and pitch-catch techniques are directly translatable for calibration of unfocused receivers. Hence, the pulse-echo technique was employed for numerical analysis and was followed by experimental calibration measurements for an unfocused transducer in Chapter 3. Numerical results demonstrated that minimal error (\( \leq \pm 1 \) dB) is obtainable using this method for calibrating the receive sensitivity of unfocused transducers, assuming that perfect alignment is obtained. By using the average pressure magnitude measured over a plane by a hydrophone as an approximation of the reference measurement, calibration measurements were shown to be accurate and less susceptible to errors induced by misalignment. Hence, the pulse-echo technique was used to calibrate the receive sensitivity of the transducer used as a PCD during sonophoresis in Chapter 2. This calibration along with the characterization of the employed receive system electronics conducted in Chapter 3 enabled the quantitative analysis conducted in Chapter 4.

In Chapter 4, a quantitative and system-independent measurement and analysis technique was developed for direct acoustic characterization of cavitation. First, this measurement approach is conducted using the techniques in Chapter 3 to calibrate
the PCD in order to convert system-measured voltages to the the cavitation-
radiated pressures incident over the receiver. Second, using the correction factor
derived in Chapter 4, the PCD-measured pressure is converted to the average
cavitation-radiated power within a defined region of interest. This correction factor
was provided as a simple analytic expression that can be obtained computationally,
requiring only the calculation of the Rayleigh integral over a discrete uniform grid
throughout a defined ROI in order to account for the relative spatial sensitivity
of the PCD to sources within its field-of-view. This approach corrects for the on-
average diffraction in the cavitation-radiated pressure field over the PCD surface.
The significance of this approach is that system-dependencies and diffraction effects
are fully removed from acoustic emission measurements made by a single-element
receiver, enabling direct comparison of results obtained using different receiving
systems, experimental configurations, or frequencies. In addition, from a dosimetry
perspective, the acoustic power radiated by cavitation within a defined ROI can be
quantified in order to assess the acoustic energy or power delivered to tissue during
therapeutic ultrasound and its relationship to the onset of associated bioeffects.

Simulations were conducted in Chapter 4 using different test configurations,
PCD geometries, and frequencies to demonstrate the feasibility of quantifying the
average acoustic powers radiated by cavitation over a ROI using the derived factor to
correct cavitation-radiated pressures measured by various PCDs. Specifically, using
simulated cavitation sources, the average radiated acoustic power was analytically
calculated and compared with corresponding simulated PCD-measured acoustic
powers before and after compensation with the derived correction factor. The
results of these simulations demonstrated that prior to compensation, the received acoustic powers from cavitation measured by different PCDs were not comparable. However, after compensation, the estimated cavitation-radiated acoustic powers were shown to be comparable between different PCDs, ROI dimensions, and frequencies. Additionally, and equally as important, the simulated PCD-measured powers after compensation provided accurate estimation of the calculated cavitation-radiated powers for all of the investigated ROI dimensions, frequencies, and PCDs.

Using the system-characterization values derived in Chapter 3 for the PCD system employed for the sonophoresis trials conducted in Chapter 2, the acoustic powers received by the employed PCD from cavitation were calculated in Chapter 4. Using these values with the derived compensation factor for the employed sonophoresis configuration, the corresponding subharmonic acoustic powers radiated by stable cavitation over the skin surface during IFS and HFS were calculated. The relationship of the radiated acoustic power values with corresponding changes in skin resistance were compared between IFS and HFS treatment regimes. For these trials, the skin resistance decrease obtained in the presence of subharmonic-producing cavitation during either treatment regime were previously shown in Chapter 2 to be comparable. However, in Chapter 4 the subharmonic acoustic power produced by cavitation over the skin surface was also shown to be equivalent among IFS and HFS treatments. Hence, equivalent acoustic powers radiated by stable cavitation were shown to correspond with equivalent changes in skin resistance between the different treatment regimes, independent of the frequency employed for treatment.
5.2 Discussion and Future Directions

In Chapter 2, the occurrence of subharmonic-producing cavitation during sonophoresis was shown to correspond with decreases in skin resistance. These findings were further explored in Chapter 4, where changes in skin resistance were found to scale with the acoustic power radiated by subharmonic-producing cavitation over the skin surface, indicating that increases in skin permeability achieved during sonophoresis are directly related to the acoustic power radiated by stable cavitation over the skin surface, independent of the frequency of ultrasound used for treatment.

The significance of these results is in the guidance they offer to future sonophoresis treatments employing IFS and HFS treatment regimes. Specifically, the goal of prospective studies should be to initiate, sustain, and maximize the occurrence of stable cavitation over the treated skin surface in order to exploit the associated bioeffects resulting in an increased rate and level of obtainable permeabilization. In order to achieve this goal, sonophoresis insonation parameters for either treatment regime may be optimized by altering the duration of the pulse and quiescent periods to sustain and maximize stable cavitation activity [Hitchcock et al., 2011]. This approach may additionally be compensated in order to increase cavitation activity at the skin surface by incorporating the use of ultrasound contrast agents (UCA) as cavitation nuclei, a strategy that has been commonly employed in other ultrasound-enhanced therapies [Price et al., 1998, McDannold et al., 2006, Datta et al., 2008], in the medium coupling the treatment transducer to skin [Park et al., 2010, Park et al., 2012]. However, because the nuclei for bubble activity originates after the UCA shell has expanded beyond its linear elastic limit and ruptured, and the liberated
gas may dissolve rapidly, sonophoresis trials may require supplementation of UCAs throughout a given trial in order to obtained sustained stable cavitation throughout the treatment [Marmottant et al., 2005, Bader and Holland, 2012]. Furthermore, this approach may be most appreciable for HFS treatments because UCAs are manufactured within a size distribution resonant to the ultrasound frequencies employed for these treatments, providing optimally sized cavitation nuclei [Klibanov, 2002, Bader and Holland, 2012].

Cavitation within the skin was also shown to contribute to permeability enhancement during HFS in Chapter 2. This was observed only for HFS trials likely because resonant bubble diameters at higher frequencies are smaller and more comparable to the dimensions of lacunar voids within the skin where cavitation can occur [Simonin, 1995, Bommannan et al., 1992, Menon et al., 1994]. For example, the resonant size of bubbles to frequencies employed for HFS are on the order of 3 $\mu$m or less, whereas the resonant size of bubbles to frequencies employed for IFS may exceed 30 $\mu$m and surpass the approximately 20 $\mu$m thick dimensions of the stratum corneum [Leighton, 1994, Polat et al., 2011]. Further complicating treatments that would be designed to initiate cavitation within the skin, especially at lower frequencies, is that the preexistence of gas bodies are typically sparse and the threshold to initiate cavitation activity is spatially variable within tissue [Fry et al., 1995]. To compensate for this challenge, one group of skin samples was purposely overhydrated with air-saturated PBS prior to sonophoresis treatment in Chapter 2. Because the hydration techniques employed in Chapter 2 may not provide a clinically viable approach, more potent and efficient sonophoresis treatments may be
more readily accomplished by promoting cavitation in the coupling medium outside of the skin in comparison to inside of skin tissue.

The accuracy of the calibration of a PCD’s receive sensitivity is fundamental to cavitation characterization as it directly influences the accuracy of measured pressures, and by extension the accuracy of estimated acoustic powers radiated by cavitation over an ROI. Hence, the requirements for accurate broadband characterization of a PCD’s receive sensitivity using various methods were investigated and developed in Chapter 3. In comparison to a bistatic scattering substitution technique [Collin and Coussios, 2011], the measured pressure waves in a pulse-echo or pitch-catch technique provide higher signal-to-noise ratio (SNR) resulting in a greater usable bandwidth for calibration, and measurements are less prone to inaccuracies due to misalignment. This is due to the incident or reflected wave measured by the receiver arriving with significantly greater amplitude than that of a pressure wave emanating from a spherical scatterer. Because the bistatic scattering substitution technique is dependent on the spherical directivity of the scattered wave relative to the receiver, the resulting measurement accuracy decreases rapidly at higher frequencies as the scattered wave becomes more complex in directivity. In addition, this approach is only applicable to calibration measurements for focused receivers and is especially prone to calibration measurement errors resulting from significant spatial averaging over the receiver surface with minor transducer misalignment. In order to extend this approach to unfocused receivers, compensation for the diffraction effects would be required following the calculations conducted in Chapter 3 and applied similarly to those employed for reciprocity.
techniques [Zhang et al., 2016]. In addition, this approach could be extended to unfocused receivers by making far-field measurements, but this would not be feasible for small scatterers.

For a pulse-echo or pitch-catch technique, effects from spatial averaging were diminished by conducting reference pressure measurements using the average magnitude of calibrated hydrophone-measured pressures instead of the complex values. In addition, this approach to making reference measurements mitigated systematic errors induced by erroneous alignment of transducers or water temperature measurements (Figure 3.9) that would otherwise lead to a reduction in the measured average complex pressure and error in distance calculations employing time-of-flight measurements, respectively. Specifically, this approximation was shown to result in reference measurements that were typically within ±1 dB (Figures 3.6 and 3.7) of the PCD-measured pressure, even with minor misalignment of the PCD, providing error that is inherent to this calibration technique.

The precision and accuracy of calibration measurements are influenced the most by reproducibility of the transducer alignment and uncertainty provided by the sensitivity of the manufacturer-calibrated hydrophone used for reference measurements. For the calibration measurements conducted in Chapter 3, PCD-generated voltages measured in a pulse-echo configuration were shown to be repeatable, with frequency-averaged uncertainties of 12.8% and 12.6% over the calibration bandwidths for an unfocused and focused PCD, respectively, over repeated measurements after reconfiguration of the transducer alignment. In comparison, the contribution from the manufacturer-calibrated hydrophone was
more significant, providing average uncertainties of 19.7% and 24.3% for those used to calibrate an unfocused and focused PCD, respectively. The uncertainty associated with the manufacturer-calibrated hydrophone sensitivity is unavoidable, but should be accounted for. Propagation of these measurable uncertainties provided a cumulative frequency-averaged uncertainty in the receive sensitivities of approximately ±2–3 dB for calibration of either transducer. This uncertainty directly propagates to measurements of cavitation-radiated pressures received by a PCD, as well as to the characterization of radiated acoustic powers following the approach developed in Chapter 4.

In Chapter 4, the theory employed to derive a correction factor accounting for diffraction effects in the cavitation-radiated field in order to estimate the cavitation radiated power within a defined ROI was tested in simulation. The results of these simulations illustrated the potential of using calibrated PCD measurements compensated by the derived factor to predict within ±1 dB the acoustic power radiated by cavitation within an area or volume. This error can be attributed to the random uniform distribution of simulated cavitation sources, resulting in random interference in the radiated pressure received by the simulated PCD that differs from estimated on-average diffraction effects provided in the compensation factor calculation. In addition, this error is assumed to be an approximate upper limit due to the simulated constraints including the low number density and coherence of sources as well as the relatively low number of independent observations that were made and averaged over.Comparatively, the uncertainty of ±1 dB between simulated PCD measurements after correction and analytically calculated acoustic
powers radiated by cavitation is within the bounds of accuracy associated with that of the measured PCD receive sensitivity in Chapter 3. However, the uncertainty in simulation results were derived using a homogeneous fluid as the propagation medium and cavitation sources modeled as discrete point sources with finite bandwidth. Furthermore, the cavitation-radiated field was assumed to propagate linearly both in simulations and for the example application for sonophoresis. For experimental and clinical applications, the sensitivity of a PCD to broadband sources generated in and emissions propagating through various heterogeneous tissues needs to be assessed.

Influences due to nonlinear propagation, attenuation, and scattering from heterogeneities in various tissue layers were neglected in the cavitation characterization studies conducted in this dissertation. However, these factors may require further investigation in future studies to identify and assess their influence on the accuracy of the cavitation characterization techniques developed in Chapter 4. In order to assess the influences of these various effects and to validate the cavitation characterization methods developed in Chapter 4, future studies could be conducted using single-bubble experiments within a viscoelastic tissue-mimicking phantom or a noncavitating acoustic sources such as a thin wire or spherical scatterer, similar to those that have been conducted for passive imaging of cavitation [Gyöngy and Coussios, 2010b] and backscatter compensation [Chen et al., 1997]. Benefiting these prospective studies would be the use of well characterized or readily characterizable acoustic sources or scatterers in order to compare experimentally measured values with known or calculated values. For
example, single bubble experiments could be conducted by directly comparing PCD-measured acoustic pressures with those predicted by numerical modeling using a modified Keller–Miksis approach, a validated model that accounts for viscoelasticity of the surrounding medium [Yang and Church, 2005, Collin and Coussios, 2011]. These comparisons could be used to investigate additional effects associated with phenomena such as attenuation, nonlinear propagation, changes in speed sound induced by tissue heating, and scattering of the treatment ultrasound beam due to random inhomogeneities in tissue. In addition, these studies should include inertial cavitation in order to assess potential effects associated with the propagation process for broadband sources and any measurement limitations due to the finite bandwidth of PCD systems.

The cavitation characterization technique developed in Chapter 4 is one potential approach to monitoring and quantifying cavitation activity that removes several limitations that have plagued traditional passive monitoring techniques by characterizing cavitation-radiated powers independently of the employed receiver or system, frequency, and treatment configuration. This approach was implemented representatively for the sonophoresis trials conducted in Chapter 2, however is extendable to other applications where passive cavitation monitoring would be conducted. For example, traditional analysis methods have used voltage signal generated by the receiving system to estimate or predict ultrasound-mediated vessel rupture [Hoerig et al., 2014], endothelial cell damage [Hwang et al., 2006], and other cellular bioeffects [Hallow et al., 2006]. The challenge with these measurements is that they are not translatable to other experimental configurations or to clinical
applications because they are specific to the employed receiving system and do not
directly characterize the cavitation ‘dose’ required to initiate the observed bioeffect.

In comparison, using the approach employed in Chapter 4, standard interactions
of the target tissue with cavitation could be established. For example, this
approach was representatively investigated in Chapter 4, illustrating a log-linear
relationship between cavitation-radiated subharmonic acoustic powers over the skin
surface and skin permeability increases. These results could provide a standard
metric for assessing the progress of sonophoresis treatments by monitoring the
cavitation-radiated powers over the skin. For other applications, this characterization
approach could be employed to identify comparable relationships between radiated
acoustic powers and associated bioeffects, which could be broadly applied by other
investigators to monitor, assess, or optimize the progress of a specified treatment.

The role of cavitation during therapeutic ultrasound is significant, especially for
enhanced drug delivery applications [Bailey et al., 2003, Pitt et al., 2004, Coussios
et al., 2007, Polat et al., 2011]. In this dissertation, the role of cavitation
during sonophoresis employing intermediate- (IFS) and high-frequency (HFS)
ultrasound was investigated and identified (Chapter 2). Aiding these findings
was the development of quantitative and system-independent measurement and
analysis techniques for characterizing cavitation activity. These approaches included
techniques for measurements of cavitation-radiated pressures received over a
transducer used as a passive cavitation detector (PCD) in Chapter 3 and a method
for characterizing cavitation-radiated acoustic powers within a treatment region of
interest in Chapter 4.
Bibliography


