INVESTIGATION INTO THE ORIGIN AND NATURE OF VARIABILITY IN QUANTITATIVE MEASUREMENTS OF TUMOUR BLOOD FLOW WITH CONTRAST-ENHANCED ULTRASOUND

by

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A thesis submitted in conformity with the requirements for the degree of Masters of Science
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Abstract

Investigation into the Origin and Nature of Variability in Quantitative Measurements of Tumour Blood Flow with Contrast-Enhanced Ultrasound

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Microbubble ultrasound (US) contrast agents have been used to monitor the progression of anti-angiogenic chemotherapies. However, US backscatter measurements used in contrast imaging are inherently variable, given the presence of many microbubbles of random position and size. A model was developed to investigate the influence of US scanner and microbubble characteristics on these variable measurements. The Coefficient of Variation was used to measure variability. It was found that an optimum excitation frequency exists that minimizes this variability. In the case of Definity™, a 2.25 MHz centre-frequency pulse yielded a less variable measurement than at 5 MHz. Conversely, decreasing microbubble concentration was found to significantly increase variability. Evidence suggests that microbubbles are no longer Rayleigh scatterers at sufficient low concentrations. Post-processing was found to aid in reducing measurement variability by averaging samples where microbubble positions are uncorrelated. As well, reduction can be achieved by averaging about a region-of-interest of uniform perfusion.
Dedication

For my family, who offered me unconditional love and support throughout the course of this thesis.
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## Symbols and Abbreviations

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<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>$2D$</td>
<td>Two-Dimension</td>
</tr>
<tr>
<td>$3D$</td>
<td>Three-Dimension</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>Gamma distribution shape parameter</td>
</tr>
<tr>
<td>$\bar{I}$</td>
<td>Sample mean intensity</td>
</tr>
<tr>
<td>$\beta$</td>
<td>Gamma distribution shape parameter</td>
</tr>
<tr>
<td>$\chi$</td>
<td>Percentage of effective dominant scatterers</td>
</tr>
<tr>
<td>$\ddot{r}$</td>
<td>Bubble shell acceleration</td>
</tr>
<tr>
<td>$\ddot{r}$</td>
<td>Bubble shell velocity</td>
</tr>
<tr>
<td>$\delta$</td>
<td>Exponent of power relationship between effective no. of scatterers and CoV</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>Exponent of power relationship between total no. of scatterers and CoV</td>
</tr>
<tr>
<td>$\Gamma()$</td>
<td>Gamma function</td>
</tr>
<tr>
<td>$\kappa$</td>
<td>Polytropic constant</td>
</tr>
<tr>
<td>$\lambda_o$</td>
<td>Center wavelength of excitation pulse</td>
</tr>
<tr>
<td>$\langle I \rangle$</td>
<td>Mean intensity</td>
</tr>
</tbody>
</table>
\( \langle I \rangle_T \) Mean intensity

\( \mu \) Mean

\( \nu \) K-distribution shape parameter

\( \nu_L \) Fluid dynamic viscosity

\( \nu_s \) Viscoelastic model shell viscosity

\( \nu_{Th} \) Fluid equivalent viscosity to thermal damping

\( \sigma \) Standard deviation

\( \sigma_s \) Backscatter cross-section coefficient

\( \sigma_T \) Total Backscatter cross-section coefficient

\( A \) Amplitude of a sum of complex exponentials (i.e. resultant/ amplitude envelope)

\( a \) Amplitude of a complex exponential

\( a_e \) Equilibrium bubble radius

\( b \) K-distribution shape parameter

\( CEUS \) Contrast-Enhanced Ultrasound

\( CI \) Confidence Interval

\( CoV \) Coefficient of variation

\( d_s \) Microbubble shell thickness

\( DEF \) Definity\(^{TM}\)

\( E () \) Expectation operator
\( f_o(MHz) \)  Center frequency of excitation pulse in units of MHz

\( FGB \)  Free Gas Bubble

\( FWHM \)  Full-Width-Half-Maximum

\( G_s \)  Viscoelastic model shell shear bulk modulus

\( I \)  Intensity of a sum of complex exponentials (i.e. intensity envelope)

\( K_\alpha() \)  Modified Bessel function of the 2nd kind, with order \( \alpha \)

\( M \)  Effective number of scatterers (in K-distribution)

\( MI \)  Mechanical Index

\( N \)  No. of independent measurements

\( n \)  Moment order

\( N_{bab} \)  No. of microbubbles

\( n_{cyc} \)  Number of wavelengths

\( N_{dom} \)  Effective no. of dominant scatterers

\( N_{tot} \)  Total no. of scatterers

\( p \)

\( p_i \)  Incident wave pressure

\( p_L \)  Pressure in the fluid medium at the shell

\( p_o \)  Hydrostatic pressure

\( PDF() \)  Probability density function

\( PP_{neg}(MPa) \)  Peak-negative pressure in units of MPa
$QCEUS$  
Quantification Contrast-Enhanced Ultrasound

$r$  
Bubble radius

$ROI$  
Region-of-Interest

$S_f$  
de Jong model lumped friction constant

$S_p$  
de Jong model lumped stiffness constant

$sgn()$  
Sign operator

$SNR$  
Signal-to-Noise Ratio

$T$  
No. of repeated trials

$V_{in-vial}$  
Withdrawn concentrated Definity$^{TM}$ microbubble volume

$V_{res}$  
Resolution cell volume

$V_{sol}$  
Withdrawn diluted Definity$^{TM}$ microbubble volume

$Var()$  
Variance operator

$x$  
Scatterer/microbubble concentration
Chapter 1

Quantitative Ultrasound

1.1 Introduction

Research in the field of ultrasound (US) imaging has significantly progressed over the past two decades with the development and commercialization of a class of US contrast agents, namely microbubbles. Second-generation microbubbles consist of a population of lipid-encapsulated perfluorinated gas bubbles. Within a fluid environment, the liquid-to-gas interface makes microbubbles ideal US scatterers. However, the spatial distribution of microbubbles is stochastic in nature. In addition, microbubble behaviour is dependent on bubble size, composition, and incident pressure waveform, and each parameter is associated with its own statistical distribution. Backscatter measurements of microbubbles are therefore inherently variable.

When injected intravenously, microbubble concentration becomes increasingly diluted while travelling through the systemic circulation. Furthermore, a wide range of microbubble concentrations are encountered, owing to differences in blood volume. Concentration can also vary significantly if the microbubble dose is transient (ex. bolus), or where microbubbles are locally burst and subsequently replenished. Given the increased use of tools to quantify microbubble concentration by measuring the microbubble backscatter
signal, better understanding of the sources of variability and a means of decreasing this variability are required. At sufficiently high microbubble concentrations, measurement variability is predictable. However, the statistical model is not applicable with fewer microbubbles. Therefore, the motivation of this thesis is to understand the statistical variability of US measurements of microbubbles at low microbubble concentrations.

1.2 Microbubble Contrast Agent

Like many other technologies, US is ever-evolving and is finding greater clinical applicability. The introduction of microbubble contrast agents is a particularly significant development as US can now image the normally acoustically-invisible vasculature.

It was first reported that small bubbles could enhance contrast in a clinical US examination when Gramiak and Shah had rapidly injected saline solution into the hearts of patients (Gramiak and Shah, 1968). They observed an increase in echoes originating at the aortic root during an echocardiogram. The strong echogenic properties characteristic of microbubbles, as well as larger bubbles and pockets of air, are a result of the abrupt change in acoustic impedance at the liquid-gas interface. Because their physical size can approximate that of red blood cells (RBCs), microbubbles can pass readily through the pulmonary circulation, as well as remain in the circulatory system. Therefore microbubbles are an effective intravascular contrast agent. However, relying on free air bubbles can pose some significant challenges, particularly a short lifetime that can be measured in seconds (Plesset and Prosperetti, 1977).

Second generation microbubbles now use perfluorinated gas (e.g. $SF_6$, perfluoropropane), and are encapsulated by a phospholipid monolayer shell (Brancewicz et al., 2006). In addition to reducing the rate of gas diffusion out of a bubble (Katiyar et al., 2009; Epstein and Plesset, 1950), an encapsulating shell offers increased stiffness as the bubble is compressed, resulting in enhanced nonlinear bubble behaviour when a bubble
encounters an incident US pressure wave (De Jong et al., 1992). This property is exploited by US contrast imaging methods. Because tissue does not ordinarily produce a nonlinear echo, detecting microbubbles within blood vessels effectively images the vascular space.

When a bubble is gently driven acoustically, the motion of its surface and the surrounding fluid oscillate like a mass on a spring, or rather a linear, harmonic oscillator, exhibiting oscillatory phenomena like resonance (Leighton, 1997). In 1917, Rayleigh derived a differential equation that could be used to model nonlinear bubble behaviour (Rayleigh, 1917). Plesset refined this model to account for the viscosity of fluid around a free air bubble, and his equation is now commonly referred to as the Rayleigh-Plesset equation (Plesset and Prosperetti, 1977). Ever since, a number of improvements and alternative derivations have been exercised, yielding a family of equations, each considered to be a modified Rayleigh-Plesset equation (Trilling, 1952; Keller and Miksis, 1980; De Jong et al., 1992; Church, 1995; Hoff, 2000; Marmottant et al., 2005).

1.3 Clinical Applicability

Clinicians have expressed increased interest in using US contrast imaging. In particular, the ability to image the vasculature and highly perfused tissues (e.g. liver, heart) using microbubbles has increased the clinical relevance of this contrast agent (Burns, 1996). In addition, contrast imaging is being used as a tool to delineate the size of tumours and monitor the effectiveness of anti-angiogenic therapies (Wilson et al., 2000). Identifying clinically-relevant parameters (e.g. blood flow, blood volume) to characterize and monitor blood flow using contrast-enhanced US (CEUS) has become a rich area of research.

One of the key strengths of CEUS is the fact that microbubbles remain in the vasculature. Given that bubbles are acoustically active for diagnostic US frequencies if their sizes lie between $1 - 10\mu m$, their targeted size distribution will preclude them from leaking into the extravascular space (Feinstein et al., 1984). As an intravascular contrast agent,
Chapter 1. Quantitative Ultrasound

microbubbles provide clinicians with the opportunity to interrogate properties associated with blood flow dynamics.

For example, the analysis of blood flow dynamics aids in the investigation of tumour growth and treatment efficacy. Tumours grow by eliciting angiogenesis (the creation of new blood vessels). Normal tissue consists of a well-organized vasculature, formed through an orchestrated release of various growth factors and signaling molecules right from embryogenesis (Alberts et al., 2002). Due to the haphazard release of growth factors by cancerous cells, a disorganized capillary network is formed around the periphery of a given tumour (Jain, 1988). The resulting vascularization can be significantly different than that of the surrounding healthy tissue. However, the extensive network of capillaries and other small vessels cannot be imaged by traditional imaging methods performed using current clinical US, x-ray, or magnetic resonance (MR) scanners due to vessel sizes of < 25µm in diameter. In the 1970s, the realization that tumours engage in angiogenesis therefore spurred a new wave of research into intravascular contrast agents that could be used to image blood perfusion (Folkman et al., 1971).

A recent emphasis on quantification of US measurements has highlighted the variable nature of microbubbles (Williams et al., 2011; Hudson et al., 2009). The fabrication of microbubbles produces a population with a variable size distribution. Further, this size distribution will change with time due to gas diffusion as well as a variety of physiological filtering mechanisms. Microbubble size is significant as bubble behaviour is a function of radius. In addition to the size of microbubbles, the concentration of microbubbles will differ from tissue to tissue, with concentrations high in organs like the liver, and concentrations low in less perfused tissues. Although, for a fixed transducer position, microbubble concentration is expected to be constant during a steady infusion of microbubbles, it is more variable during dynamic injections like a bolus. Quantification will be further elaborated in §1.5.

In in vivo conditions, other forms of variability exist for which clinicians must mini-
mize. Significant motion artifacts can be introduced by breathing and the pulsatile motion of the heart. Significant work has been done to introduce in-plane motion correction while out-of-plane motion is reduced via a strategic orientation of the US probe (Williams et al., 2011). Breath-holds can be requested in order to eliminate breathing motion temporarily. US probe position and orientation must also be controlled carefully to ensure that the tissue-of-interest does not move within the US field.

1.4 Physiological Concentrations

Although a single dose of microbubbles may be administered, the microbubble concentration encountered in different tissues can vary greatly. Dose administration, physiological factors, and the vascularity of tissue can significantly affect the resulting in vivo concentration. Although a sufficient concentration of microbubbles is necessary in order to achieve fully developed speckle and relate US image intensity to microbubble concentration, it is often the case that either the tissue vascularity is too sparse, or the dose is too little, challenging the validity of this criterion.

A dose of concentrated or diluted microbubble solution is typically delivered to the site-of-interest via a superficial intravenous injection. Previous clinical studies have chosen to either deliver microbubble dosage in either the form of a bolus or a steady infusion (Hudson et al., 2009; Lassau et al., 2011). However, all clinically approved microbubble products have a specified maximum dose for a given examination. Definity™, for example, can be delivered up to 10µl/kg as an intravenous bolus (Stapleton et al., 2006). Note that the recommended dose is per unit mass of the patient.

To reach the tissue of interest, microbubbles must take a path that impacts their spatial and temporal distribution. Microbubbles must first circulate to the heart, through the pulmonary capillary network, before being distributed throughout the systemic circulatory system. With each organ acting to modulate the dose, the transit time of the
incoming microbubble bolus is particularly affected. The heart acts as a mixing chamber, diluting the bubble concentration. The lungs subsequently filter larger bubbles as large bubbles become lodged within the narrow capillaries and creating temporary pulmonary occlusions. However, there is no evidence of microbubble trapping, vascular occlusion, or tissue damage (Keller et al., 1987) in the process of microbubble administration. Lastly, pulsatile cardiac output further modulates the temporal distribution of microbubbles.

A number of other physiological factors can influence local microbubble concentration. On average, an adult will have 5l of blood, although soon after intravenous injection, microbubbles are only significantly diluted by the time they reach the heart. The total and ventricular blood volume are patient-specific. In addition, cardiac output is modulated by sympathetic and parasympathetic hormones based on an individual’s health and emotional condition. Further, systemic blood pressure changes and constricted or dilated arteries can influence the blood volume and corresponding number and rate of microbubbles entering the tissue-of-interest.

Over time, microbubble concentration is influenced by passive and active mechanisms that act to clear the agent. Passively, gas within a given microbubble will diffuse into the plasma. The use of perfluorinated gas in microbubbles was chosen for its biocompatibility as well as the relatively lower diffusion rate. For a 2.5µm dia. Definity™ microbubble in an air-saturated medium experiencing a surface tension of 25mN/m, the dissolution time can be 45mins (Sarkar et al., 2009). A more rapid rate of clearance is due to the active endocytosis of microbubbles by macrophages as blood is filtered through the spleen and kidneys (Mattrey et al., 1987; Goldberg et al., 1994). Microbubbles will continue to recirculate and be detected throughout the body until either gas diffusion results in a globule of lipid, and macrophages of the reticuloendothelial system remove them from circulation.

Even given a steady cardiac output, the microbubble concentration within different organs can be different. Blood vessel organization differs from tissue to tissue based on
organ function and nutrient intake requirements. For example, as compared to limp, unused muscle tissue, the cortex of the kidney will show a higher concentration of microbubbles given that the body's blood supply must pass through the nephritic glomeruli to be filtered. Larger blood vessels like arteries and veins can be easily delineated as their sizes exceed the resolution of the US system. However, often it is the vasculature of tissue parenchyma that is of interest in microbubble studies. Given the relatively low resolution of clinical US scanners, there can be many individual capillaries within a single voxel supplying in the voxel many more parenchymal cells. For these areas-of-interest, the actual blood vessel volume is significantly impacted by the density of small blood vessels and capillaries.

Although difficult to perform, absolute blood volume measurements have been made, particularly in the kidneys where QEUS is often performed. Assuming a bolus injection of Definity™ microbubbles is administered, a maximum microbubble concentration can be estimated for non-cancerous renal tissue. Although each kidney represents 0.2 % of total body weight, a single kidney can have a blood flow rate of 550 ml/min or 11 % of cardiac output (Guyton, 1981). However, blood flow rate and mean transit time must both be known in order to arrive at an approximation to blood volume. Through an indicator-dilution method, Logan et al. (1973) estimated an average renal blood volume for hypertensive males to be $35 \pm 9$ ml. This was corrected for a body surface area of $1.73 \text{ m}^2$. Consider that the end-diastolic volume can be up to 120 ml, and the stroke volume output of the heart is typically about 70 ml (Guyton, 1981). If a 0.2 ml bolus of Definity™ microbubbles is delivered (Williams et al., 2011), with an average microbubble in-vial concentration of $3.87 \times 10^9$ bubbles/ml (see Table 2.2), assuming complete mixing by the time the blood reaches the left ventricle during diastole and uniform perfusion of the kidney, the maximum in-blood microbubble concentration at the peak of this bolus in a hypertensive kidney is $2.8 \times 10^6$ bubbles/ml. If microbubble concentration is a measure of the proportion of microbubbles per unit volume of kidney tissue, and the
average kidney has a volume of $187 \pm 21 \text{ ml}$ \cite{Bakker1999}, then at the peak of a bolus, the maximum microbubble concentration would be $5.3 \times 10^5$ bubbles/ml. However, a bolus typically has a mean transit time, indicating the microbubbles have become diluted by the time they reach the tissue-of-interest. In addition, a bolus measurement will show a slow and gradual decrease in microbubbles, with a small, periodic signal increase, indicative of recirculation \cite{Goldberg1994}. Therefore the microbubble concentration encountered will range from zero to the previously calculated maximum concentration.

1.5 Quantifying Microbubbles

The use of microbubbles has been motivated by the capability of the agent to enhance the vasculature during traditional B-mode US imaging. However, the preferred means of imaging microbubbles have been to use schemes devised to suppress partially or completely the signal from tissue while enhancing the conspicuity of the microbubble signal. As a consequence of tissue suppression, these alternative schemes allow image data to be directly related to microbubble concentration. However, care must be exercised during the design and execution of any US study destined for quantification. The measured microbubble response is sensitive to a number of US scanner parameters. As well, the nonlinear relationship between the microbubble response and these parameters make it difficult to compensate for changes in these parameters once a study has been initiated. This thesis will focus on the variable response of microbubbles using the B-mode imaging scheme, although future work can apply the same methodology to determine the sensitivity of CEUS variability to other frequency regimes.

B-mode imaging relies on the acquisition of echoes from adjacent columns of tissue (A-mode) to form an image. Each A-mode acquisition sends a pressure wave into the depths of the tissue, with echoes recovered from the various depths and subsequently
Figure 1.1: Simulated A-line of a 5MHz backscattered signal. The measured RF signal, amplitude envelope, and intensity envelope are shown.

post-processed. Figure 1.1 shows such a receive signal, as well as an envelope drawn about the signal. The echo intensity envelope is derived by squaring the amplitude envelope. Each echo intensity envelope is then scaled and compressed, and displayed as a line of grey-scale pixels, for easy visualization. This is done by calculating the envelope of the signal and compressing the linear scale into a log scale, because the strength of the signal from various tissues (and microbubbles) differ in orders of magnitude. If an US wave is produced about a given fundamental frequency, B-mode imaging is sensitive to a bandwidth centered about the fundamental frequency, and therefore can be referred to as first-harmonic imaging.

Contrast Imaging is an extension of the traditional B-mode imaging scheme. Contrast Imaging encompasses many methods with which to detect the signal from microbubbles, while suppressing the signal from tissue. One such method is Harmonic Imaging. The transducer can be made sensitive to a bandwidth about a particular harmonic other than the fundamental frequency. Harmonic Imaging, in particular, is sensitive about the 2nd harmonic, with one further post-processing step added to the B-mode processing chain. If microbubbles can be made to behave nonlinearly, then the scattered pressure wave will contain many harmonics, as seen in Figure 1.2. Harmonic Imaging methods then filter everything but a bandwidth about the 2nd harmonic and produce an image based on
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Figure 1.2: Simulated microbubble RF frequency response given 5MHz centre-frequency, 5 cycle, 75$kPa$ amplitude pulse excitation, for a 1.6$\mu m$ radius free gas bubble (FGB), a 1.6$\mu m$ rad. Definity$^{TM}$ bubble (DEF), and a 0.3$\mu m$ rad. Definity$^{TM}$ bubble.

This filtered signal.

More advanced contrast imaging methods utilize multiple transmit pulses to tease out the nonlinear properties of microbubbles. Such multi-pulse sequences include Pulse Inversion (Hope Simpson et al., 1999) and Amplitude Modulation (Brock-Fisher et al., 1996; Eckersley et al., 2005). In both examples, the additive response from scatterers can be identified as linear or nonlinear by using excitation pulses of differing amplitudes. Non-zero additive backscatter signals inherent to microbubbles lie at the heart of contrast imaging methods employed in today’s clinical scanners.

However, due to the nonlinear response of microbubbles, image acquisitions are sensitive to changes in US scanner parameters. US imaging provides a wealth of parameters that can be adjusted to improve resolution, Signal-to-noise ratio (SNR), and depth penetration, to improve image quality. However, in addition to image considerations, parameters must be chosen conservatively to avoid microbubble disruption. On the other hand, initial parameters should be selected to elicit the best SNR. If the data are to be used for quantification studies, chosen parameters need to be specified with care and consistently
for the duration of a study.

Microbubbles can provide a means to image solely the vasculature. However, care must be taken when selecting appropriate scanner settings, with particular consideration for microbubble disruption and SNR. This thesis will focus on an evaluation of CEUS variability in 1st-harmonic imaging, as an important first step. The additional complexity associated with understanding the variability inherent in the nonlinear response of microbubbles at frequencies above the fundamental will be addressed as future work.

1.6 Measurement Variability

Putting aside physiological considerations, a number of factors contribute to the variability of US measurements made within the clinic and on the benchtop. The coefficient of variation (CoV), defined as the ratio between standard deviation-to-mean, will be used as a metric of measurement variability. Factors that are of considerable interest are those that reduce the CoV. Intrinsic variability is closely associated with microbubble concentration, whereas extrinsic variability can be associated with output transducer intensity, frequency, and geometry.

1.6.1 Spatial Distribution

Microbubbles are discrete, nonlinear scatterers that are typically distributed throughout the volume-of-interest. Therefore, their relative distribution will influence the phase at which individual scattered waves return to the transducer, and the net interference pattern that is measured at the transducer face. It is typically assumed that backscatter measurements adhere to Rayleigh statistics, but there are actually a number of factors to consider before this conclusion can be made.

At concentrations that result in a large number of microbubbles within a US resolution cell, the overall scattering response is indistinguishable from a continuous medium. The
resolution cell volume is approximated as the length of an US pulse times a circular area with a diameter equal to the full-with-half-maximum (FWHM) of the beam profile of the transducer at the focus. This is illustrated in Figure 1.3. At lower concentrations, the response from individual bubbles can easily be identified in a given US measurement, as interference effects are minimized.

A reasonable starting point is to consider that microbubbles are distributed uniformly. Microbubbles move within an enclosed capillary network in vivo, however cells are usually within tens of micrometres from a capillary as dictated by the oxygen diffusion length (Kayar and Weiss, 1992), indicating the density of the vasculature is sufficient to allow many microbubbles to occupy the a given US resolution cell while being restricted to the vascular space. Furthermore, there are many bifurcations leading up to and from the capillaries, so there are many paths that microbubbles can take.

A uniform spatial distribution is also a sensible assumption based on the limited microbubble interaction. Phospholipid-coated microbubbles are not expected to aggregate together because they are composed of amphipathic molecules with a lipid head group that preferentially orients away from the watery environment, as well as shielded by its hydrophilic moiety (Lozano and Longo, 2009). Polyethyleneglycol (PEG) is a very hy-
drophilic polymer that is bound to a lipid, forming a lipopolymer, and ensuring solubility and dispersion of liposomes in a water-rich medium. In addition to conferring water soluble properties to an otherwise hydrophobic spherical shell, a long PEG molecule can be used to create sufficiently large spheres of hydration to further reduce the chance of interaction with other bodies (Alberts et al., 2002). The use of PEG reduces the effect of Gibbs free energy-related attractive/repulsive forces on the spatial distribution of microbubbles.

Regardless of the spatial distribution, some microbubbles will be insonated and detected preferentially over others. The acoustic field distribution of the transmit and receive transducers are not uniform, but rather consists of a focal zone, a main lobe, as well as sidelobes, of increased pressure and sensitivity. However, given the size range for microbubbles, typical concentrations, echogenicity, as well as the length scale for the -6dB width and depth-of-field of the main lobe that defines the resolution cell volume, a sufficient number of microbubbles will be present within the sample volume to produce a measurable signal.

The spatial distribution of microbubbles are not impacted very much by buoyant forces. Assuming the bubbles have reached terminal velocity, as drag forces eventually oppose the upwards force by the surrounding liquid, 1µm diameter bubbles will rise as fast as 3.5µm/s while 10µm diameter bubbles can rise up to a rate of 350µm/s (Epstein and Plesset, 1950). If the sample volume is not further mixed, or replenished with new bubble solution, a size gradient will emerge with bubbles with increasing diameter found closer to the top.

Lastly, within the presence of a sound beam, bubbles have the propensity to move towards one another. It is conceivable that interference created between 2 scattering microbubbles creates a pressure differential that draws them closer together. This force is referred to as the Bjerknes force (Bjerknes, 1906; Crum, 1975). However, due to the constant flow of microbubbles, a given pair of microbubbles are unlikely to spend enough
1.6.2 Bubble Size Distribution

Although clinical microbubbles are considered micron-sized, they typically do not have a uniform equilibrium radius. The manufacturing process plays a very significant role in the microbubble size distribution. Given that many microbubbles are either formed immediately before use (Goertz et al., 2007) or re-activated (Madjar et al., 1993), bubble handling can also influence the size distribution. Being aware of the microbubble diameter is important because the size of the microbubble determines the echo strength and the acoustic scattering cross-sectional area.

Figures 1.4 shows a typical size distribution for Definity\textsuperscript{TM} microbubbles. This sample has been activated from a new vial. The activated vial was allowed to sit for 15 minutes after activation, shaken for 10s, and allowed to settle for 30s before the size measurement was made. Goertz et al. show that waiting varying lengths of settling time can result in different distributions resulting in different acoustic properties (e.g. attenuation) (Goertz et al., 2007).

Using a viscoelastic model, Gorce et al. show that although bubbles smaller than
1\mu m can compose the majority of a bubble population, the remaining larger bubbles contributed the most to the backscatter signal \cite{Gorce et al., 2000}. Due to the fact that smaller diameter bubbles have resonant frequencies that typically exceed diagnostic US frequencies, their individual scattering is quite weak.

In addition, large linear scatterers were shown to scatter more strongly, and simulations showed a better correspondence between acoustic cross-section with respect to total surface area and total volume, as compared to total number.

The nonlinear response of microbubbles impacts the variable scattering response of a population of microbubbles. Commercially available microbubbles have a polydispersed size distribution. Because lipid-coated bubbles radially oscillate like a damped oscillator, each bubble radius is associated with a resonance frequency. Therefore, a certain subpopulation of microbubbles will scatter far more effectively than others. The number of effective scatterers within a given resolution cell then depends on the concentration of the subpopulation of bubbles and specific resonance characteristics.

Calculating the total backscatter cross-section can provide a means of justifying why the resulting backscatter echo is greater for one excitation frequency over another. The formulation that Church uses for the total backscatter cross-section is as follows \cite{Church, 1995}:

\[ \sigma_{\text{tot}} = \sum_{r=a_1}^{a_2} \sigma_s(a_e) \ast n(a_e) \]  
\[ \text{where } \sigma_s(a_e) = \frac{4\pi}{I_o} \int_0^T r(t)^2 \ast p(a_e, t) \ast \dot{r}(a_e, t) dt \]  
\[ \text{where } I_o = \frac{|p_i|}{2\rho c} \]

where \( a_1 \) and \( a_2 \) are the bubble radius size distribution limits, \( a_e \) is the nominal bubble radius, \( r \) is the instantaneous bubble radius, \( I_o \) is the incident echo intensity, \( p_i \) is the incident pressure amplitude, \( p(a_e, t) \) is the instantaneous scattered pressure at the surface, while \( \dot{r}(a_e, t) \) is the instantaneous rate of change of bubble radius.

A further complication in predicting total backscatter cross-section is the dynamic
nature of microbubble size. Bubble sizes are time-dependent as they shrink due to gas diffusion. Gas will inevitably diffuse out of a microbubble because a higher internal pressure is required to oppose surface tension as well as the ambient pressure \cite{Epstein1950}. Although, a bubble coating significantly decreases surface tension from that of a free bubble \cite{Meltzer1982}. As well, todays microbubbles use heavier gases (e.g. SF$_6$, C$_3$F$_8$) to reduce the rate of diffusion \cite{Mattrey1987}. However, for phospholipid bubbles Marmottant identifies a radius at which point the bubble shell buckles, reducing surface tension to zero. Buckling is the process by which the lipid shell slips over itself. He suggests that the loss of the inner and outer pressure differential could be one reason why phospholipid microbubbles may remain viable for a significant amount of time after activation \cite{Marmott2005}.

### 1.7 Modeling a Microbubble Population Response

A computer model is required to explore the factors that may impact the variability of contrast-enhanced US quantification measurements involving microbubbles. Although the theoretical basis for acoustic measurements of microbubbles is well established, a complete numerical simulation is not trivial to implement. Acoustic production, propagation, scattering, and reception of US will have to be considered carefully.

The following section is devoted to describing the relevant processes that will have significant bearing on any simulation investigating the variable acoustic response from microbubbles.

#### 1.7.1 Prior Computational Studies

Research within the past 30 years has unraveled the complexities of single microbubble behaviour, enabling the prediction of single microbubble scattering. A number of papers have attempted to model the response of a microbubble population with limited success.
Especially due to the computational demands of modeling many individual bubbles, certain simplifications have had to be made.

The majority of microbubble studies place an emphasis on single microbubble behaviour, and often show this behaviour change with respect to bubble radius, or other single parameters. To consider the effects of a population, some authors consider the superposition of measurable quantities, allowing attenuation and backscatter coefficients to be estimated. Usually, a population of bubbles is only considered in order to arrive at some meaningful way to associate attenuation measurements with ensemble microbubble shell properties. (De Jong and Hoff, 1993; Church, 1995; Hoff, 2000). However, to properly estimate the variability in the resultant backscatter signal, individual microbubble backscatter signals must be calculated.

Most papers describing statistical simulations pertain to characterizing of tissue using US image characteristics (Hong et al., 2005; Shankar, 2000; Dutt and Greenleaf, 1994; Roberjot et al., 1996). Therefore, the backscatter solution can be arrived at through convolution rather than resorting to a nonlinear solver due to the linear behaviour of cells. Often, these simulations are limited to at most 2-dimensions, as the argument is often that the 3D case is an extension of the 2D case. Unfortunately, when the sample volume of the scatterers is oblique in 3D, then results from a 3D analysis can diverge from a simplified 2D geometry.

Hibbs et al. (2007) (Hibbs, 2007) came closest to a true simulation of microbubbles distributed in 3D space. However, to simplify the number of calculations required in the simulation, the same radius was assumed from bubbles within a $x^{1/3}$-wide cubic voxel, where $x$ was the number of microbubbles per millilitre. In addition, microbubbles were positioned along a fixed grid spacing, albeit that the grid spacing was fine enough to ensure interference. To account for the random spatial distribution and size distribution of microbubbles, a fixed grid spacing and similarly-sized neighbouring bubbles likely bias the statistics of the resultant backscatter signal at the transducer.
In this present work, parallel computational resources have been exploited to enable a more rigorous determination of the characteristics of a population response under various conditions. Microbubbles will be randomly-distributed. Therefore, a model that replicates more realistic wave interference by microbubbles is realized.

1.7.2 Microbubble Behaviour

Accurately modeling the nonlinear behaviour of microbubbles is essential when investigating the backscatter response to various US conditions. The evolution of the nonlinear differential equation used to describe the radial excursion of the bubble wall has led to a number of forms that are applicable under various conditions.

The initial mathematical groundwork for bubble oscillation was established by Besant and Rayleigh. It was Reynolds who first postulated how bubbles could produce sound by acting as a resonator \( \text{(Reynolds, 1901)} \), but it was Minnaert that established the mathematical formalism and validated it experimentally \( \text{(Minnaert, 1933)} \). During the latter half of the 20th-century, Plesset and Prosperetti greatly furthered understanding of bubble behaviour with respect to sound production \( \text{(Prosperetti and Lezzi, 1986)} \). The Rayleigh-Plesset model now has become the starting point for most discussions on microbubble behaviour. Once the medical imaging community found coated microbubbles to be a favourable US contrast agent, modified Rayleigh-Plesset models by de Jong and Church were proposed to account for shell effects \( \text{(De Jong et al., 1992; Church, 1995)} \). Hoff further revised the Church model, now to be referred to as the viscoelastic model \( \text{(Hoff, 2000)} \). Most recently, Marmottant was able to account for the presence of more subtle effects, and is considered to have produced the most accurate microbubble model available \( \text{(Marmottant et al., 2005)} \).

The viscoelastic model, as defined by Hoff, is considered a suitable model for low
amplitude insonation of coated microbubbles. This equation is defined by Equation 1.4.

\[ r \dddot{r} + \frac{3}{2} \dot{r}^2 - \frac{p_L - p_o - p_i}{\rho} - \frac{r}{\rho c} \dot{p}_L = 0 \]  

where \( r \) is the bubble radius, \( p_L \), the liquid pressure at the bubble surface, \( p_o \), the hydrostatic pressure, \( p_i \), the incident pressure, \( \rho \), the density of water, and \( c \), the speed of sound in water. Further, \( p_L \) has been defined to be:

\[ p_L = -4 (\nu_L + \nu_{Th}) \frac{\dot{r}}{r} - \Delta T_s + p_o \left( \frac{a_e}{a} \right)^{3\kappa} \]  

where \( \Delta T_s = 12 \left( \frac{d_s}{r_e} \right) \left( \frac{1}{8} G_s \left( 1 - e^{-8\dot{r}} \right) + \nu_s e^{-4\dot{r}} \right) \)

where \( \nu_L \) is the dynamic fluid viscosity, \( \nu_{Th} \), the equivalent fluid viscosity due to thermal damping, \( \kappa \), the polytropic constant, \( d_s \), the shell thickness, \( G_s \), the shell shear modulus, and \( \nu_s \), the shell viscosity.

Marmottant acknowledges that his model reduces to the viscoelastic model if: (1) the bubble radius does not reduce below the buckling radius, and (2) the radial excursion is small compared to the bubble radius. These conditions are satisfied. Therefore, the viscoelastic model is used to predict microbubble behaviour in the following analysis. Specifically, because a low output transducer intensity is typically used in vivo, a mechanical index of \( \leq 0.05 \) (\( MI = \frac{PP_{neg}(MPa)}{\sqrt{f_o(MHz)}} \)) is chosen to prevent microbubble destruction. Using frequencies of \( \leq 5 MHz \), this translates into a peak-negative incident pressure of:

\[ PP_{neg}(MPa) = MI \cdot \sqrt{f_o(MHz)} \]  

In order to arrive at the scattered pressure, the following equation can be used:

\[ p(R,t) = \rho_o \left( \frac{r^2 \ddot{r} + 2r \dot{r}^2}{R} \right) \]  

1.7.3 Wall Effects

Microbubble behaviour can also be modulated by the proximity of the capillary vessel wall. In an unbound medium, surrounding fluid moves radially as the bubble expands
and contracts. However, with a cylindrical, compliant vessel wall, outward fluid flow is impeded and redirected (Wu, 2002). In addition, changes in ambient pressure follow as the redirected fluid couple the vessel wall with the bubble. It has been found that the resonance frequency is decreased under such conditions (Hosseinkhah and Hynynen, 2011). Wall effects can significantly impact the frequency-dependent population response. Unfortunately, due to complex fluid interactions, most research has involved the finite element analysis of a vessel with a single bubble and no single-variable analytical model is available. However, experimental conditions have been defined such that wall effects do not require consideration.

### 1.7.4 Population Response

Microbubble measurements are seldom a measurement of the response of 1 or 2 microbubbles, but rather a response derived from a great number of bubbles (i.e. $10^8$ bubbles/ml) within a given sample volume. Most commercial agents have a polydispersed size distribution and are distributed randomly in space. The randomness of certain bubble properties lead to predictable statistical properties of the resulting signal. The variability inherent in CEUS measurements is a function of these random variables, and is analyzed in-depth in this thesis.

The resultant signal measured at the transducer surface is a result of the superposition of individual microbubble echoes. If each microbubble is insonated by an incident pressure wave, then each microbubble becomes a tiny US point source as they scatter and re-radiate acoustic energy in all directions. However, it is typically assumed that waves from neighbouring microbubbles do not excite and therefore produce secondary scattering. This is referred to as the Born approximation (Cobbold, 2007).

However, interference of randomly-positioned linearly scattering particles is expected. In an A-line, as well as in a B-mode image, this interference is known as speckle. Wave theory suggests that each wave will superimpose with each other, producing constructive
and destructive interference. Once the signal has propagated to the receive transducer, this interference results in pressure amplitudes that result in a Rayleigh-distributed pressure envelope. Hence, data adhering to this assumption is considered to follow Rayleigh statistics. A further calculation of the intensity envelope will show more broadly varying undulations as compared to the centre-wavelength of the transmit beam, characteristic of speckle. See Figure 1.1. As a consequence of this statistical behaviour, the apparent increase and decrease in intensity does not necessarily indicate a change in concentration of scatterers.

Given its statistical origin, a signal demonstrating fully-developed speckle has predictable statistical properties, including the variability of the signal intensity. A relatively small number of scatterers is required to achieve fully-developed speckle. The size of the full-with-half-maximum of the broad undulations, or so-called speckle grains, is in fact a function of the properties of the imaging system as well as the spatial distribution of the scatterers. If the scatterers remain stationary, then the same speckle pattern will persist. However, the specular nature of the measured signal can be reduced if multiple measurements can made over the same scan plane, but with the scatterers sufficiently displaced with respect to the transducer, or vice versa, such that new speckle patterns form. Averaging over these images will smoothen the speckle and yield an intensity that is proportional to the number of scatterers. Also, the variability of the resulting averaged measurement will be reduced.

Microbubbles may or may not adhere to Rayleigh statistics depending on local acoustic conditions. Under low amplitude conditions, a microbubble will scatter proportionally with incident pressure amplitude, and will behave like a linear scatterer. However, microbubbles also resonate at frequencies corresponding to their size and composition. The resulting frequency dependency, which itself is dependent on bubble size, will yield a response from a microbubble population that can be highly frequency-dependent, as well as sensitive to its size distribution.
As a last consideration, the wide range of \textit{in vivo} microbubble concentrations can result in situations where there is an insufficient number of scatterers within a resolution cell to warrant Rayleigh statistics. Measurements of very low concentrations of microbubbles could even show the response of individual microbubbles, due to a complete absence of wave interference. In fact, strong resonance of a few microbubbles in a relatively concentrated solution can result in a significantly larger contribution to the resultant waveform on receive, resembling that of a sparse concentration scenario. On the other extreme, a high microbubble concentration can yield fully-developed speckle with an expected variability, although attenuation effects would reduce the mean echo intensity. Non-Rayleigh statistics can yield significantly unpredictable results resulting in greater variability.

### 1.8 Statistical Concepts

Scattering of energy by a system of particles is a ubiquitous problem in classical physics, and a theoretical framework exists in the form of a series of proposed statistical models. Various statistical metrics can be used to characterize a distribution. Of particular interest will be the expectation value, or mean, as well as the coefficient of variation (CoV), which is a relative measure of the dispersion of the data with respect to the mean,

\[
\text{CoV} = \frac{\sigma}{\mu} \tag{1.9}
\]

where \(\sigma\) is the standard deviation and \(\mu\) is the mean of a particular quantity. The CoV is a non-dimensional metric, allowing easy comparison between the variability of different parameters.
1.8.1 Rayleigh Statistics

Consider a uniform spatial distribution of identical, linear scatterers, small in size and in-abundance within a thin-shelled hemispherical voxel, whose maximum microbubble separation, $\Delta r$, in the radial direction with respect to the transducer is less than the length of the excitation pulse, $n\lambda_o$, where $n$ is the number of cycles and $\lambda_o$ is the centre wavelength of the excitation pulse. Because $\frac{\Delta r}{n\lambda_o} \leq 1$, the scattered waveforms arriving at the transducer face will interfere.

Rayleigh described an analogous scenario with respect to vibrations along a string [Rayleigh, 1880], and suggested that because the phase of each individual vibration was uniformly-distributed, each with an amplitude, $a$, the resultant could have real and complex components that are themselves Gaussian-distributed. Upon a change of variables, the probability distribution function (PDF) of the amplitude of the resultant, $A$, has the form outlined in Equation 1.10.

$$PDF(A) = \frac{2A}{\langle I \rangle} e^{-\frac{A^2}{\langle I \rangle}}$$  \hspace{1cm} (1.10)

If intensity is taken to be $I = A^2$ then the corresponding PDF($I$) is given as an exponential PDF, as defined in Equation 1.11.

$$PDF(I) = \frac{1}{\langle I \rangle} e^{-\frac{I}{\langle I \rangle}}$$  \hspace{1cm} (1.11)

Because the mean, $\mu$, and standard deviation, $\sigma$, of Equation 1.11 is equal to the mean echo intensity for a given point, $\langle I \rangle$, then $CoV = 1$. This reflects a rather large variability. Henceforth, scatterers that adhere to the assumptions held by Rayleigh will be referred to as Rayleigh scatterers, and the corresponding statistical model will be referred to as the Rayleigh statistical model (sometimes referred to as Rayleigh statistics). In addition, the unity CoV will be referred to as the Rayleigh limit.

The amplitude of the resultant is equivalent to a single instance in time of the amplitude envelope of a detected US signal. If the assumptions governing Rayleigh statistics hold, then a single instance in time of the corresponding intensity envelope will be
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This thesis will compare statistical attributes of experimental and simulated US backscatter measurements with expected statistical properties associated with the Rayleigh statistical model.

1.8.2 Sample Mean Statistics

A signal measured at the transducer face, produced by incoherent scattering, and approaching characteristics of Rayleigh statistics will yield a variability in intensity that will asymptotically converge to the Rayleigh limit. This implies that there is a 66% probability that a given intensity measurement will take on a value ranging from 0 to twice the mean echo intensity. Averaging multiple independent measurements within a given ensemble can improve the estimate with tighter 95% confidence intervals. Consider that the exponential distribution is a special case of the Gamma distribution,

\[ \text{PDF}(I) = \frac{1}{\Gamma(\alpha)\beta^\alpha} \left( I^{\alpha-1} e^{-\frac{I}{\beta}} \right) \quad (1.12) \]

for \( I \geq 0 \), where \( \alpha = 1 \) and \( \beta = \langle I \rangle \). The mean and variance of Gamma distribution is defined to be \( \mu = \alpha \beta \) and \( \sigma^2 = \alpha \beta^2 \), leading to a mean and variance of an exponential distribution to be \( \langle I \rangle \) and \( \langle I \rangle^2 \), respectively. The sample mean echo intensity is defined as:

\[ \bar{I} = \frac{1}{N} \sum_{i=1}^{N} I_i \quad (1.13) \]

where \( N \) is the size of the sample over which the average is taken and each sample is expected to have an identical expected intensity, \( \langle I \rangle \). Using the Method of Moment Generating Functions [Wackerly et al. 2007], the PDF of the sample mean echo intensity (\( \bar{I} \)) then becomes:

\[ \text{PDF}(\bar{I}) = \frac{N^N}{\Gamma(N) \langle I \rangle^N} \left( \bar{I}^{N-1} e^{-\frac{N\bar{I}}{\langle I \rangle}} \right) \quad (1.14) \]

where \( \alpha = N \) and \( \beta = \frac{\langle I \rangle}{N} \) when compared with Equation 1.12. The mean and variance for the sample mean echo intensity then becomes \( \langle \bar{I} \rangle \) and \( \frac{\langle I \rangle^2}{N} \), respectively. Subsequently,
Figure 1.5: Averaging $N$ exponentially-distributed variables results in a CoV that is equal to $\frac{1}{\sqrt{N}}$. Error bars represent 95% confidence intervals.

the CoV becomes $\frac{1}{\sqrt{N}}$. Figure 1.5 shows the relationship between the sample size and the CoV. The 95% confidence intervals have been produced by using an appropriate gamma-distributed random number generator for $N = 1000$ instances.

Within the clinical context, acquiring many independent measurements is easy when microbubbles are delivered as a constant infusion and the US probe position is fixed. Typically the pulse repetition rate is low enough that a new microbubble spatial distribution is realized from frame-to-frame. However, incremental reduction in CoV becomes increasingly more expensive as a 95% confidence interval with an approximate CoV of 0.5 and 0.35 requires a minimum of 33 and 129 independent measurements, respectively, to be averaged (Jacobsmeyer 2007). Under other systemic and local dosage schemes such as bolus injection and disruption-replenishment of microbubbles, it is not possible to gather multiple independent measurements as the microbubble concentration is changing too rapidly.
1.8.3 Averaging over a Region-of-Interest

An important step in current Quantification Contrast-Enhanced Ultrasound (QCEUS) studies is the definition of a region-of-interest (ROI) within an US image. Here, the number of microbubbles within the defined area is of great interest, and pixel intensity within the ROI is averaged to arrive at a single averaged intensity. Given that an US B-mode image is composed of a number of scan-converted A-lines, an analogous 1D ROI is the time window.

Taking the average over an intensity envelope within a time window will yield a windowed time-average intensity. Scan conversion aside, this is analogous to the average intensity derived from an ROI analysis. Although points can themselves be exponentially distributed, adjacent points are correlated with each other. The minimal distance between 2 uncorrelated points, or the correlation length, is equal to the duration of the pulse used to excite the microbubbles. As the sampling frequency is high enough to finely discriminate the centre-frequency components of the receive signal, adjacent points are well within the correlation length, and therefore these points are not statistically independent from one another. Consequently, the variability of the windowed time-average intensity cannot be expected to decrease by $\frac{1}{\sqrt{N_{\text{pts}}}}$ where $N_{\text{pts}}$ is the number of points in the time window.

However, averaging across an intensity-envelope time window to arrive at the mean echo intensity of the ensemble may be justified if the intensity envelope is considered an ergodic process. In signal processing, the ensemble average, $\langle I \rangle$, often must be approximated from a single instance of the ensemble. However, strict criteria must be adhered to before a waveform is considered ergodic. The windowed time-average intensity can be expressed as,

$$\langle I \rangle_T = \frac{1}{T} \int_{-T/2}^{T/2} I(t) \, dt$$

(1.15)

where $T$ is the length of the time window. A process is considered ergodic if the limit as $T \to \infty$ of the mean, variance (square of the standard deviation), and autocorrelation of
the windowed time-average intensity satisfy the following:

\[
\lim_{T \to \infty} E \{ \langle I \rangle_T \} = \langle I \rangle \tag{1.16}
\]
\[
\lim_{T \to \infty} Var \{ \langle I \rangle_T \} = 0 \tag{1.17}
\]
\[
\lim_{T \to \infty} E \{ R_T \} = R \tag{1.18}
\]
\[
\lim_{T \to \infty} Var \{ R_T \} = 0 \tag{1.19}
\]

where \( E \{ \} \) is the mean operator, \( Var \{ \} \) is the variance operator, and \( R \) is the autocorrelation of the intensity.

Although it is not expected that a windowed time-average intensity will decrease as \( \frac{1}{\sqrt{N_{pts}}} \), it is expected that the larger the time window, the more accurate the estimation of the windowed time-average intensity is to the ensemble average. Consequently, a decrease in CoV is expected.

### 1.9 Thesis Overview

A significant problem faces clinicians and researchers interested in using microbubbles for CEUS quantification studies. There is the lack of understanding as to what US parameters can yield the least variable measurement. Because CEUS is being used to derive physiologically-relevant parameters, understanding the error attributed to the US measurement is important.

This thesis proposes that variability in backscatter measurements of microbubbles is a function of characteristics of a microbubble population as well as the US system used to interrogate the microbubbles. Furthermore, this variability can be minimized through an appropriate selection of parameters. Specifically, this thesis will interrogate how microbubble concentration and US frequency can influence variability. A model will be validated and used to identify a clear relationship between variability of a given measurement and the aforementioned parameters.
Chapter 2

A Statistical Analysis of the Microbubble Population Response

2.1 Specific Aims

Ultrasound imaging of the microvasculature can be significantly improved by using a contrast agent consisting of micrometre-sized, encapsulated bubbles called microbubbles. Presently, microbubbles are being used in research hospitals around the world to monitor the extent of therapeutic response of anti-angiogenic cancer treatments. Identifying responders and non-responders can help develop a more rigorous treatment schedule. However, measurements must be sufficiently accurate and reproducible if they are to be used to influence decisions regarding patient care.

Multiple scenarios can be encountered where a low concentration of microbubbles is present within the tissue-of-interest. Whereas measurement statistics can be predicted from speckle statistics arising from high concentrations of contrast agent, measurements arising from low concentrations have not been properly addressed.

The motivation of this thesis is to understand the statistical variability of US measurements of microbubbles at low microbubble concentrations. Established scattering
theory predicts the statistical characteristics of the backscatter of multiple scatterers, although it is not clear how microbubble backscatter characteristics may differ. A computer model has been developed to simulate the acoustic conditions encountered by a microbubble population, microbubble behaviour during excitation with ultrasound, as well as the measured net backscatter response. The model is validated by experiment, and is then used to investigate the influence of experimental parameters on the relationship between the variable microbubble response and concentration. Both experiment and simulation can help identify operating conditions in which prevailing backscatter theory may not be applicable.

2.2 Hypothesis

It is hypothesized that the variability of measurements of US contrast agents has its origins in both population characteristics of microbubbles and the acoustic parameters of an US system, and these may be optimized so as to minimize this variability.

The manner in which this thesis aims to address the aforementioned hypothesis will be to:

- create a computer model to simulate US contrast measurements and compare with measurements from physical experiments,
- measure the effect of varying microbubble concentration, US frequency, and measurement processing,
- and draw conclusions based on statistical metrics.

2.3 Methods

Pulse-echo experiments were conducted to investigate the variability of backscatter measurements from populations of microbubbles. A computer model was created to emulate
acoustic conditions and to generate similar backscatter measurements. To validate the computer model, experimental results were compared with simulation using the same experimental parameters.

Acoustic conditions were fixed and a range of low microbubble concentrations were considered. Given the frequency-dependent response of microbubbles, the experiment was repeated for pulses with 2 different centre-frequencies. The experiment is first described, followed by details regarding the model. The manner in which the data is post-processed and statistically quantified is subsequently explained.

2.3.1 Experimental Design

An appropriate number of measurements needed to be collected to determine the statistical characteristics of microbubble US backscatter measurements under various acoustic conditions. Given the frequency-dependence of the microbubble response, 2 different transmit pulse centre-frequencies were considered (i.e. 2.25 MHz, 5 MHz). At each frequency, 6 different microbubble concentrations were used \( (N_{\text{conc}}=6) \). See Table 2.1. In order to minimize experimental bias, the order of selected microbubble concentrations was chosen in a pseudo-random manner. Random high and low concentrations were alternated. As well, to verify that time had a minimal impact on pre-diluted microbubble characteristics for the duration of each experiment, measurements were repeated using another pseudo-random order using the same vial. For each microbubble concentration considered, 100 statistically-independent pulse-echo measurements \( (N_{\text{meas}}=100) \) were made. Each series of experiments was repeated 5 times in order to reach statistically significant conclusions \( (T=5) \). This corresponded to the use of 5 new vials of Definity\(^{TM}\). The results then underwent similar post-processing to that of a clinical scanner in B-mode.
2.3.2 Microbubble Agents

Definity\textsuperscript{TM} (Lantheus Medical Imaging, North Billerica, MA) is composed of micron-sized phospholipid-coated bubbles with a perfluoropropane gas core, and is approved for clinical use in Canada. Activation of Definity\textsuperscript{TM} microbubbles was a relatively simple process. Once a vial of Definity\textsuperscript{TM} was removed from the freezer and kept at room temperature for 30 min, the VialMix\textsuperscript{TM} microbubble activator was used to shake the vial rapidly at a pre-determined frequency for 45 s. The vial was then left for 30 min to cool down to room temperature, a process that allowed the shell properties and size distribution to stabilize. After the 30 min duration, every withdrawal from the vial was preceded by a gentle, thorough shaking for 10 s and the vial is upturned for 30 s. The solution was withdrawn from the upturned position. Given that the total amount of agent consumed was less than 200 \(\mu\text{l}\), no venting was utilized to minimize displacement of the perfluoropropane gas with air.

After activation of Definity\textsuperscript{TM} and before the start of experiments, the size distribution was measured using a Beckman Coulter Multisizer\textsuperscript{TM} 3 machine. For Definity\textsuperscript{TM}, the size distribution was only ascertained after waiting 30 min after microbubble activation. The rubber-sealed vial was shaken for 10 s before withdrawal, and allowed to sit for 30 s in an upturned position. Previous lab tests showed that the Multisizer 3\textsuperscript{TM} provided reliable measurements when an absolute bubble count between 100,000 and 400,000 was made. Consequently, though trial-and-error, 10 \(\mu\text{m}\) of Definity\textsuperscript{TM} was withdrawn from the vial, diluted in 10 ml of Beckman Coulter Isoton II diluent, and 50 \(\mu\text{l}\) were withdrawn by the Multisizer\textsuperscript{TM}.

To control the concentration of microbubbles being interrogated as well as achieve very low concentrations, multiple dilutions of the initial concentrated microbubble solution were required. The Coulter Counter\textsuperscript{TM} was used to determine the size distribution of the agent before the start of each experiment. Given that microbubbles with diameters ranging from 1-10 \(\mu\text{m}\) have a resonance frequency close to the clinical diagnostic
frequencies considered, the number of bubbles was reported to be the summation of all
the microbubbles with diameters ranging between 1-10 \( \mu m \).

If \( V_{in-vial} \) \( \mu l \) of in-vial microbubble solution is withdrawn, diluted into 10ml of Isoton,
then \( V_{sol} \) \( \mu l \) of this dilute solution is withdrawn, and for \( N_{bab} \), the number of bubbles
counted between 1-10 \( \mu m \), the concentration of the in-vial microbubble solution is deter-
mined to be:

\[
C_{definity} \ (bubbles/ml) = N_{bab} \frac{10^3 \ \mu l}{V_{sol} \ \mu l} \frac{1}{V_{in-vial} \ \mu l} \frac{1000 \ \mu l}{1 \ ml} \frac{N_{bab} \ast 10^7}{V_{sol} \ast V_{in-vial}}
\]  

(2.1)

To dilute Definity\( ^TM \) to achieve very low concentrations, 2 dilution steps were re-
quired. On average, \( C_{definity} \approx 4 \times 10^9 \) \( \text{bubbles/ml} \). \( S \) \( \mu l \) was first withdrawn from
the vial, which was then diluted into 50 ml of deionized gas-equilibrated water. Once
well-mixed, \( T \) \( \mu l \) was then withdrawn from the dilute sample, and mixed thoroughly, but
gently, into a 1 l gas-equilibrated, deionized water reservoir.

The final microbubble concentration can be expressed in a number of ways. See
Table 2.1. The dilution ratio is the volume of concentrated Definity\( ^TM \) per unit volume
of deionized, gas-equilibrated water, and is the preferred unit of concentration in a clinical
or preclinical context. The number of microbubbles per resolution cell volume quantifies
the number of bubbles expected to be within the focal volume of the transducer and that
have the potential to dominate the backscatter measurement.

2.3.3 Linear Scatterers

The use of linear scatterers is expected to result in an increase in CoV with a decrease in
concentration. A solution of Orgasol\( ^TM \) has been previously used as a blood-mimicking
fluid for Doppler US experiments, and was an ideal candidate for a homogeneous solu-
tion of narrow-size distribution scatterers \[Rammarine et al., 1998; Thorne et al., 2008\].
Orgasol\( ^TM \) itself is composed of ultrafine polyamide particles, of which a sample was
graciously provided by Dr. Tamie Poepping (University of Western Ontario). In order to
Table 2.1: Targeted simulated and experimental microbubble concentrations expressed in absolute terms (bubbles per unit volume), in terms of the ratio between volume of concentrated Definity™ and volume of water (dilution ratio), and in terms of the number of microbubbles within the focal volume which is a function of the excitation pulse characteristics (i.e. resolution cell volume). Physical experiments only considered 6 microbubble concentrations, marked with (†).

<table>
<thead>
<tr>
<th>Bubbles per Unit Volume (bubbles/ml)</th>
<th>Dilution Ratio ((×10^{-6} : 1))</th>
<th>Number of Bubbles per 2.25MHz Resolution Cell Volume</th>
<th>Number of Bubbles per 5MHz Resolution Cell Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>0.0065</td>
<td>0.44</td>
<td>0.040</td>
</tr>
<tr>
<td>50 †</td>
<td>0.013</td>
<td>0.88</td>
<td>0.080</td>
</tr>
<tr>
<td>75</td>
<td>0.020</td>
<td>1.3</td>
<td>0.12</td>
</tr>
<tr>
<td>100 †</td>
<td>0.026</td>
<td>1.8</td>
<td>0.16</td>
</tr>
<tr>
<td>300</td>
<td>0.078</td>
<td>5.3</td>
<td>0.48</td>
</tr>
<tr>
<td>500 †</td>
<td>0.13</td>
<td>8.8</td>
<td>0.80</td>
</tr>
<tr>
<td>1000 †</td>
<td>0.26</td>
<td>18</td>
<td>0.16</td>
</tr>
<tr>
<td>2000</td>
<td>0.52</td>
<td>35</td>
<td>3.2</td>
</tr>
<tr>
<td>3000 †</td>
<td>0.78</td>
<td>53</td>
<td>4.8</td>
</tr>
<tr>
<td>4000</td>
<td>1.0</td>
<td>70</td>
<td>6.4</td>
</tr>
<tr>
<td>5000 †</td>
<td>1.3</td>
<td>88</td>
<td>8.0</td>
</tr>
</tbody>
</table>
prepare 1 l of Orgasol$^{TM}$ solution, to be simply referred to as Orgasol$^{TM}$, the following ingredients were used:

- 838.6 g water
- 100.6 g glycerol
- 33.6 g Dextran D4876, from Leuconostoc mesenteroides, average mol wt 100,000–200,000 (Sigma Aldrich, St. Louis, MI)
- 18.2 g Orgasol 2001 UD NAT (5 $\mu$m dia.) (Arkema Inc., King of Prussia, PA)
- 9.0 g JetDry (Reckitt Benckiser, Slough, UK)

The preparation of Orgasol$^{TM}$ was not trivial. Although the particles had themselves a narrow size distribution, the particles tended to aggregate, even though a surfactant was used. Subsequently, multiple mechanical filtration using a 40 $\mu$m sieve was used. A sieve of smaller grid spacing was not available at the time of preparation.

Since backscatter intensity is proportional to the number of scatterers, to the geometric cross-section, and the frequency raised to the power 4, it is not surprising that very little signal could be measured using a 2.25MHz centre frequency pulse, let alone at low concentrations. A single trial at 5 MHz was executed ($T=1$) in order to demonstrate the validity of the methodology in light that theory already predicts a deviation from Rayleigh statistics for decreasing concentrations of linear scatterers.

### 2.3.4 Flow Cell Setup

The primary role of the flow cell was to physically constrain the diluted microbubble solution in a control volume. The particular flow cell used in experiment was designed and created by Charles Darveau-Tremblay. Rather than using epoxy to cement the Mylar$^{TM}$ windows to the edges of the flow cell, a rubber seal and equally-spaced machine screws hold the Mylar$^{TM}$ in place and creates an airtight seal that can withstand >20
Figure 2.1: Gravity-driven flow setup. Dilute microbubble flow is continuous to ensure new microbubbles replenish the field-of-view of the transducer between acquisitions.

kPa gauge pressure. This design is robust against high transient hydrostatic pressures and minimizes the risk that experiments will be perturbed by a leaking flow cell.

See Figure 2.1 for an illustration of the microbubble path. During the setup of the flow cell, it was critical to ensure that no air bubbles obstructed the pathway, particularly within the flow cell, valves, and at tube connections. The flow cell was permanently oriented at 45° from the axial direction of the transducer. This minimized multiple reflections of US waves between the flow cell and the transducer face. A magnetic stirrer was placed in the flow cell and turned at 180 rpm to help ensure a well-mixed solution. A dilute microbubble solution was located in the reservoir, a fixed height above the flow cell. Solution flowed continuously through the acrylic tubing to replenish the field-of-view within the flow cell of the transducer with fresh microbubbles between consecutive acquisitions. A flowmeter was carefully monitored and the flow rate was controlled via a manual valve, while the height of the drainage outlet and reservoir water level controlled the hydrostatic pressure within the flow cell. The flow rate varied between 110-120 ml/min, due to the varying height of the reservoir water level.
2.3.5 Transducer Setup

An Olympus-Panametrics single-element spherical transducer was used instead of an array transducer typically found on clinical US scanners. A single-element transducer is the most elementary means to transmit and receive US signals, and has a well-defined, circularly-symmetric beam profile. As such, the number of transducer parameters is reduced. During the course of the experiment, 2 transducers were used. Both transducers had a focal length of 2” and a diameter of 0.75”. The centre-frequency of each transducer was 2.25 MHz (533 kHz bandwidth) and 5 MHz (1.27 MHz bandwidth) respectively.

See Figure 2.2 for an illustration of the signal pathway. The Tektronix AWG5012 arbitrary waveform generator (AWG) was controlled via a General Purpose Interface Bus (GPIB) interface using a custom, unified Labview graphical user interface. A pseudo-narrowband pulse was specified, along with the necessary gating signal for the amplifier and the digital acquisition card (DAQ) trigger. The inverted output of the specified AWG pulse was fed into the AR 50 dB pulse amplifier. The amplified signal was then conditioned by a 20 dB attenuator, a 500 kHz high pass 4th-order Butterworth filter, and a 7.5 MHz low pass 5th-order Butterworth filter, before entering the expander-limiter that then directed the signal to the transducer. On receive, the measured signal was conditioned by a 6 dB attenuator, 23 dB pre-amplifier, followed by a 10.7 MHz low pass filter, before reaching the Gage CompuScope 1602 data acquisition card (DAQ). The AWG also produced a trigger signal that was utilized by the digitizer.

Using the AWG, a rectangular-windowed sinusoidal pulse was defined for each transducer, with a pulse length of $5\lambda$. Using the same electrical configuration and transducer setup, a hydrophone was used to calibrate the output voltage with respect to the pressure at the focus of the beam. Consequently, the appropriate AWG output voltage was selected to produce a peak negative pressure of 75 kPa at the focus of each transducer.

Figure 2.3 presents the waveform and Figure 2.4 presents the excitation pulse frequency characteristics at the acoustic focus, respectively, for the 2.25MHz and 5MHz
Figure 2.2: Signal pathway used to excite and detect signal to/from transducer. (a) Schematic, (b) Actual set-up.
Chapter 2. A Statistical Analysis of a Microbubble Pop. Response

Figure 2.3: The waveforms for the excitation pulse at the focus due to a 5-cycle transmit pulse by both the 2.25MHz and 5MHz transducers using a 2.25MHz and 5MHz centre-frequency, respectively.

transducers. The FWHM bandwidth of each excitation pulse was 370kHz and 990kHz, respectively.

To ensure a reproducible setup, the single-element transducer was lined up with a vertical wire target using a series of pulse-echo measurements. The construction of the wire target and flow cell ensured that the wire target lies along the central axis of the flow cell. Using the same signal pathway and AWG output, the transducer was displaced laterally and axially with the help of a precision 3-axis positioning system. Lining up the wire target within the transducer’s acoustic focus produced a maximum pulse-echo signal from the wire target, and ensured that the flow cell was positioned at the focus.

2.3.6 Measurement Protocol

A strict control of the flow of microbubble solution was required to guarantee the acquisition of statistically-independent measurements. A reservoir, situated at a fixed height from the flow cell, was filled with 1-litre of gas-equilibrated, deionized water. The means by which the microbubble concentration was controlled is described in § 2.3.2. This
concentrated bubble solution was then diluted into the reservoir and mixed gently but thoroughly. The flow valve was opened and the reservoir continuously emptied with an initial flow rate of 120 ml/min. A magnetic stirrer was used to maintain a homogeneous concentration of microbubbles in the reservoir. As the water level decreased, the flow rate dropped to a minimum of 110 ml/min. The dilute solution entered the acoustically transparent flow cell from the bottom of a side wall, while an outlet was situated at the top of the same side wall. A flowmeter was attached downstream to the flow cell in order to monitor for flow obstructions, and the solution finally exited, at a predetermined height relative to the reservoir, into a drain. The flow valve was closed before the reservoir was sufficiently depleted to introduce air bubbles into the flow pathway.

A number of further steps were taken to ensure the appropriate data collection. A waiting period of 100 s was required from the time the flow valve is opened, to ensure that the bubble concentration within the flow cell had reached a steady state concentration matching that of the bubble concentration within the reservoir. The volume within the

Figure 2.4: The power spectrum for the excitation pulse at the focus due to a 5-cycle transmit pulse by both the 2.25MHz and 5MHz transducers using a 2.25MHz and 5MHz centre-frequency, respectively.

![Graph showing power spectrum](image-url)
A Statistical Analysis of a Microbubble Pop. Response

Chapter 2.

flow cell was insonated by the single element transducer through acoustically-transparent Mylar\textsuperscript{TM} windows. For each measurement, 100 traces were made 10 ms apart. In addition, multiple measurements were made 3 s apart to ensure new microbubbles occupied the focal volume. The custom Labview software unified control over the AWG as well as the onboard Gage CompuScope 1602 acquisition card. The software was responsible for saving the data as well as the acquisition parameters into a single binary file for later post-processing.

To make further measurements using a different concentration of microbubbles, the reservoir is rinsed and filled with 1-litre of fresh deionized water. To minimize the potential for bias, small and large microbubble concentrations are considered alternately.

Over a limited time period, different volumes of concentrated bubble solution was diluted into a fresh reservoir of gas-equilibrated, deionized water. The process of rinsing, pouring, diluting, and acquiring 100 acquisitions took \(<10\) min. Because previous lifetime measurements demonstrated the viability and consistency of Definity\textsuperscript{TM} from 15 min to 3 hr after activation, there was enough time and enough solution to consider 6 different concentrations, twice. Each measurement is repeated in order to quantify whether there is a significant difference in user-handling and agent size distribution with respect to time. A pseudo-random order was used to ensure that each concentration was utilized in the 1st and 2nd half of the experiment.

2.4 Model

MATLAB\textsuperscript{TM} (MathWorks, Natwick, MA) was used to develop a computer model to simulate the backscatter measurement of a population of microbubbles. As in §2.3.1, the simulation was executed multiple times, for the 2.25MHz and 5MHz case, as well as for multiple concentrations. See Table 2.1. Using the same parameters between experiment and simulation provided a means of validating the model. The model was then executed
for similar microbubble concentrations but simulating 20,000 independent measurements to arrive at more improved statistical metrics.

### 2.4.1 Virtual Geometry

The physical geometry of the transducer and flow cell setup was replicated in a virtual space. The FieldII Ultrasound Simulation Program is a MATLAB\textsuperscript{TM}-based program designed to calculate the impulse response for a vast range of transducer geometries (Jensen, 1996) (Jensen and Svendsen, 1992).

Once the transducer geometry is defined, FieldII is used to find the impulse response at a multitude of grid points within the volumetric bounds of the flow cell. Due to the excessive time and memory requirements needed to calculate the impulse response at the position of each microbubble, the impulse response was precalculated at the vertices of a 3D grid defined throughout the sample volume. The grid spacing was smaller than the wavelength of the US frequencies considered. The uniform spatial distribution of the virtual microbubbles was defined by the random coordinate positions of the microbubbles within this volume. Using a nearest-neighbour algorithm, the closest grid point to a particular microbubble was identified, and its impulse response was assumed to correspond to that of microbubble position. However, the phase delay for the impulse response was matched precisely with the position of the microbubble, to ensure that scattering is incoherent.

### 2.4.2 Modelling Microbubble Behaviour

The Modified Rayleigh-Plesset equation, as defined by Church and further developed by Hoff, was the nonlinear differential equation used to model microbubble behaviour (Church, 1995; Hoff, 2000). Based on the viscoelastic shell from which it is derived, Hoff’s model is also referred to as the viscoelastic model. The algorithm used to solve the nonlinear equation was derived from Bubblesim, a MATLAB\textsuperscript{TM}-based program (Hoff et al., 2000).
Chapter 2. A Statistical Analysis of a Microbubble Pop. Response

A number of input parameters are required to solve the viscoelastic model. Based on the bubble characterization of Sonazoid™ by Hoff (Hoff, 2001), the measurements were assumed to take place under isothermal conditions, leading to a polytropic constant of 1. The density of water was 1000 kg/m³, the viscosity of water was 1 mPa·s, as well, the speed of sound in water was assumed to be 1500 m/s. The ambient pressure was assumed to be 1 atm. Lastly, the bubble shell parameters needed to be specified. The viscoelastic model originally incorporated the following bubble parameters: shell thickness, shear bulk modulus (\(G_s\)), and shell viscosity (\(\mu_s\)). However, alternative microbubble parameters have been formulated by de Jong (i.e \(S_p, S_f\)), and many microbubble formulations such as Definity™ have been characterized extensively using these parameters. Although \(S_p\) and \(S_f\) can be easily related to the original viscoelastic parameters (Goertz et al., 2007), as can be seen in equation 2.2 there is a dependence on bubble radius that proved problematic when implementing the parallel nonlinear solver used. Fortunately, the viscoelastic model is amenable to substitution with \(S_f\) and \(S_p\), that allowed it to become independent of bubble radius.

\[
G_s = \frac{S_p}{6d_s} \quad (2.2)
\]

\[
\mu_s = \frac{S_f}{48\pi d_s} \quad (2.3)
\]

Faez et al. (2011) determined the \(S_p\) and \(S_f\) for Definity™, over the 7.5-15 MHz range, to be 1.64 N/m and 0.15*10^{-6} kg/s, respectively.

Bubblesim uses MATLAB™’s default nonlinear solver to predict the radial excursion of the bubble boundary within a computational time of approximately 5 s (dual-core processor, multi-threading enabled). However, given the largest number of bubbles that has been considered (\(B \approx 10000\)), and the number of virtual measurements that was needed to determine the statistical properties (\(N_{meas}=100\)), it would take \(~60\) days to complete a run. Using traditional means, it seemed unreasonable to proceed with the simulation using the default nonlinear solver.
Due to the vast number of bubbles needed to be considered, a parallel nonlinear solver was implemented, via a port of the LSODA algorithm to the NVIDIA CUDA™ programming language. Using the GPU computing capabilities of the NVIDIA GTX460™ graphics card, each solution could be derived in \(\sim 6\) ms on average, allowing the 10000*100 solutions to be calculated in \(\sim 1\ h\ 45\ min\). To ensure a robust solution, regardless of the frequency or amplitude of the incident pressure, Bubblesim would normalize time and pressure, solve using a normalized implementation of the modified Rayleigh-Plesset equation, and then scale the solution appropriately. The same normalized equation was incorporated into the GPU solver. Given the same input parameters, the GPU solver was verified to produce the same output as Bubblesim. Using the CUDA algorithm, the only deviation in output as compared to its Matlab™ equivalent, resulted from instantaneous changes in input pressures, where MATLAB™’s adaptive time-stepping nonlinear solver can handle such severe changes in input more accurately. However, given that the microbubbles were driven at low incident pressures, such behaviour was not expected nor observed.

### 2.4.3 System Dependencies

A number of system-dependent effects needed to be taken into account by the computer model, as they could not be decoupled from the experimental data. These effects included the electromechanical transfer function, attenuation, and electronic noise.

Each transducer had a characteristic frequency response curve which impacted the resulting transmitted pressure and received voltage waveform. The particular transducers used were narrowband. In a purely linear system, the transducer’s electromechanical transfer function could be deconvolved twice from the received signal, to remove dependency of the results on the characteristics of a particular transducer. However, microbubbles respond nonlinearly, even under the moderately low pressures used. This made it impractical to separate the electromechanical transfer function from experimen-
tal measurements. For each transducer, a quartz plate was used to measure the reflected pulse-echo signal of a single-cycle pulse. The voltage waveform at the terminal between the limiter/expander and transducer was measured at excitation and reception, and was deconvolved to determine the electromechanical transfer function. The model incorporated this transfer function on both transmit and receive.

Attenuation is typically frequency-dependent, and can vary based on the medium that the US wave is propagating through. Attenuation due to propagation through water was taken into account by the model.

Electronic noise was contributed by each electrical component defined in Figure 2.2. Even the electronic filters that were used to remove spurious frequency components beyond their low- or high-pass frequency threshold could have introduced noise into their passband. The statistical characteristic of this noise was determined by evaluating background measurements. However, noise was not introduced into simulated results when comparing experiment and simulation. Instead, the influence of noise was studied separately by adding noise of increasing amplitude to simulated results.

2.4.4 Post Processing Overview

Quantification of clinical data is typically performed on the intensity envelope of the backscattered signal. In accordance with B-mode imaging, a bandpass filter was applied about the fundamental frequency for each trace. Similar to drawing a ROI around a 2-dimensional B-mode image, a window-of-interest, or time window, was defined about a single A-mode trace, and the statistical properties of the intensity envelope was evaluated. Due to the similarity in form between the simulated and experimental data, the same processing pathway was used regardless of whether the data was derived from model or experiment. One-hundred (100) statistically-independent pulse-echo traces ($N_{\text{meas}} = 100$) were analyzed for each concentration considered.

To remove noise as well as limit the scope of the analysis to the fundamental frequency
regime, a 5-th order Butterworth bandpass filter was applied to all waveforms before further post-processing. The lower and upper frequency limits of this bandpass filter were 0.5 and 1.5 times the pulse centre frequency, respectively. These symmetric limits were defined in order to minimize contributions by 2nd harmonic frequency components.

The Hilbert transform was used to perform the envelope detection. However, due to an edge-tapering artifact, a larger-than-required window was necessary when executing a Hilbert transform. The amplitude data (A) was previewed, a priori, to identify an appropriate time window corresponding to the limits of the flow cell boundaries, and centred about the peak, corresponding to the acoustic focus. The Hilbert transform then produced an amplitude envelope by taking the Fast Fourier Transform (FFT) and multiplying a phase shift kernel before applying an inverse FFT. Equation 2.4 best describes the Hilbert function, where the transform adds a phase of $\pi/2$ to positive frequency components ($\omega > 0$), and subtracts a phase of $\pi/2$ for negative frequency components ($\omega < 0$). The $sgn()$ function evaluates to $-1$ if the argument is negative, and $+1$, otherwise.

\[
G(\omega) = -jsgn(\omega)F(\omega) \tag{2.4}
\]

The intensity envelope ($I$) was calculated as the square of the amplitude envelope ($I = A^2$). A smaller time window was then chosen, whose length was a function of the pulse length, and centred about the focal depth. Similar to the selection and averaging over a ROI, a windowed time-average intensity was calculated over the windowed intensity envelope ($\bar{I}$). This will be referred to as the Mean Statistic. A single point in the middle of the time window was also chosen, and this will be referred to as the Point Statistic. The data set corresponding to the mean statistic and point statistic was then used for statistical analysis.

The CoV is a primary means to evaluate the deviation from Rayleigh statistics. As mentioned in §1.8, the CoV is a ratio of the standard deviation over the mean. That is,
the CoV for any given point along the intensity envelope produced by a sufficient number of Rayleigh or Rayleigh-like scatterers is equal to 1 ($CoV = 1$). A non-unity CoV will be an indicator of non-Rayleigh statistics. For each concentration, the CoV of the mean statistic and point statistic across all the traces ($N = 100$) is computed.

## 2.5 Results

### 2.5.1 Contrast Agent Repeatability

Table 2.2 lists the in-vial Definity$^{TM}$ microbubble concentrations encountered before the start of each experiment, based on a 1-10 µm size distribution.

<table>
<thead>
<tr>
<th>Agent</th>
<th>2.25MHz expt. (bubbles/ml)</th>
<th>5MHz expt. (bubbles/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vial 1</td>
<td>4.10e9</td>
<td>3.83e9</td>
</tr>
<tr>
<td>Vial 2</td>
<td>4.04e9</td>
<td>3.72e9</td>
</tr>
<tr>
<td>Vial 3</td>
<td>3.88e9</td>
<td>3.74e9</td>
</tr>
<tr>
<td>Vial 4</td>
<td>3.92e9</td>
<td>3.85e9</td>
</tr>
<tr>
<td>Vial 5</td>
<td>3.86e9</td>
<td>3.74e9</td>
</tr>
</tbody>
</table>

Table 2.2: In-vial Definity$^{TM}$ microbubble concentrations measured before each experiment.

### 2.5.2 Size Distribution

Figure 2.5 shows the average size distribution, expressed as bubble diameter vs. bubble count, diameter vs. total bubble volume, and diameter vs. total bubble surface area, for the Definity$^{TM}$ microbubbles. Figure 2.6 also depicts the size distribution of a suspension of Orgasol$^{TM}$ solid particles considered. As is considered typical, Definity$^{TM}$ can be seen to have a peak diameter in the volume plot of $\approx 1\mu m$, with a corresponding peak in the
surface-area plot.

The Definity\textsuperscript{TM} size distribution defined in Figure 2.5 is used to determine the scattering cross-section as a function of Definity\textsuperscript{TM} microbubble radius, as well as total scattering cross-section as a function of centre-frequency of a 5\(\lambda\)-long pulse. The total scattering cross-section is defined by Equation 1.1. These results are presented in Figures 2.7a & 2.7b.

For each vial, data for a given concentration was collected twice, once in the first half of the experiment, and once during the latter half of the experiment. A Friedman test (\(N_{\text{meas}}=100\)) was applied to the 5\(\lambda\)-window-averaged mean echo intensity to test if time influenced the experimental data collected, accounting for changes in concentration. For each vial considered, window-averaged intensity for each trace was paired with the corresponding window-averaged intensity in the same-concentration experiment. The null hypothesis was that there would be no difference between early and late window-averaged mean intensities within a given vial. A contradiction to the null hypothesis would require \(p<0.05\). See Table 2.3 for the resulting p-values. These results lead to a mixed conclusion. The window-averaged mean echo intensity for most of the experiments are not significantly influenced by early and late measurements, while experiments using 3 vials in particular seemingly are significantly affected.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Freq. (MHz)</th>
<th>Vial 1</th>
<th>Vial 2</th>
<th>Vial 3</th>
<th>Vial 4</th>
<th>Vial 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definity</td>
<td>2.25</td>
<td>0.5472(\dagger)</td>
<td>0.4949</td>
<td>0.3544(\ddagger)</td>
<td>0.8761</td>
<td>0.3158</td>
</tr>
<tr>
<td>Definity</td>
<td>5</td>
<td>0.3296(\star)</td>
<td>0.7692</td>
<td>0.3861</td>
<td>0.7898</td>
<td>0.6576(\dagger)</td>
</tr>
</tbody>
</table>

Table 2.3: P-values reported for a Friedman test, testing for any significant differences between early and late window-averaged mean echo intensity experimental measurements (\(N_{\text{meas}}=100\)). Since \(p<0.05\) is considered significant, time dependency is not found to be significant. (\# of columns: \(N_{\text{conc}}=6\), except where \(\dagger N_{\text{conc}}=5\), \(\ddagger N_{\text{conc}}=4\), \(\star N_{\text{conc}}=3\))

A Friedman test was also applied to the 5\(\lambda\)-window-averaged CoV to test if time influ-
Figure 2.5: Definity™ size distribution using the following representation: (a) number vs. microbubble radius, (b) total surface area vs. radius, (c) total volume vs. radius.
Figure 2.6: Orgasol\textsuperscript{TM} size distribution using the following representation: (a) number vs. microbubble radius, (b) total surface area vs. radius, (c) total volume vs. radius.
Figure 2.7: Theoretical Definity\textsuperscript{TM} and free bubble total backscattering cross-section based on measured Definity\textsuperscript{TM} size distribution: (a) total scattering cross-section vs. microbubble radius by a subpopulation for a given radius bubble, (b) total scattering cross-section vs. pulse centre-frequency.

enced the calculated CoV. The null hypothesis was that there was no difference between early and late window-averaged CoV resulting from either changes in concentration or changes in vial. See Table 2.4 for the resulting p-values. Once outliers were identified, there appears to be insufficient evidence to conclude that the data is significantly different whether it is collected close to the beginning or end of the experiment.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Freq. (MHz)</th>
<th>Concentration Effects ($N_{meas}=6$)</th>
<th>Vial Effects ($T=5$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definity 2.25</td>
<td>0.4446\textsuperscript{†}</td>
<td>0.7839\textsuperscript{†}</td>
<td></td>
</tr>
<tr>
<td>Definity 5</td>
<td>0.9979</td>
<td>0.7459</td>
<td></td>
</tr>
</tbody>
</table>

Table 2.4: P-values reported for a Friedman test, testing for any significance effects of microbubble concentration and changes in vial to the window-averaged CoV experimental measurements (\textsuperscript{†}lowest concentration considered an outlier and omitted).
Figure 2.8: Example of Definity\textsuperscript{TM} microbubble simulated backscatter traces: (a) at 50 bubbles/ml concentration, (b) at 5000 bubbles/ml concentration. Note the differences in vertical scale.

### 2.5.3 Statistical Measures

The range of microbubble concentrations considered include on one extreme, the presence of a single dominant scatterer in the focal volume, and on the other, sufficient number of scatters to result in Rayleigh scattering. Figure 2.8 displays 3 statistically-independent backscatter traces at 50 bubbles/ml and 5000 bubbles/ml concentrations. Each trace is centered about the acoustic focus.

For the Definity\textsuperscript{TM} and Orgasol\textsuperscript{TM} experiments, Figures 2.9 and 2.10 show the mean echo intensity and CoV of the point statistic with respect to microbubble concentration. To evaluate the statistical properties of backscatter for each concentration, 100 independent backscatter measurements were made. Given that each Definity\textsuperscript{TM} experiment was repeated 5 times (T=5), each data point represents the mean of the mean echo intensity, as well the error bars represent the standard error of the mean. For Orgasol\textsuperscript{TM} mean echo intensity results, the error bars represent 1 standard deviation. Results pertaining to the 2.25MHz and 5MHz experiments have been shown on the same graph for comparison.

Experimental Definity\textsuperscript{TM} results show a linear relationship between microbubble con-
Figure 2.9: Definity\textsuperscript{TM} bubble experiments (point statistic, N=100, T=5) using a 75-kPa, 5-wavelength pulse: (a) Mean echo intensity vs. concentration, (b) coefficient of variation vs. concentration.
Figure 2.10: Orgasol™ particle experiment (point statistic, N=100, T=1) using a 75-kPa, 5-wavelength pulse: (a) Mean echo intensity vs. concentration, (b) coefficient of variation vs. concentration.

Concentration and mean echo intensity, although the backscatter was greater for 2.25 MHz excitation than at 5 MHz. Experiment also demonstrates a monotonically decreasing relationship between concentration and CoV, with the exception of the lowest microbubble concentrations. Results from 2.25 MHz excitation also shows a consistently lower CoV.

The single-trial experimental Orgasol™ results also show a similar linear relationship and decreasing trend between concentration and mean echo intensity and CoV, respectively.

In order to validate the model, simulated results were produced under the same test conditions and by producing the same number of independent backscatter measurements as per experiment. Figure 2.11 shows the corresponding mean echo intensity and CoV for the point statistic with respect to microbubble concentration. The simulated experiments were conducted using Definity™ shell parameters. Definity™ results were also computed five times (T=5) and averaged, as per experiment.

Simulated Definity™ results show a similar linear relationship between concentration and mean echo intensity although the slopes for the 2.25 MHz and 5 MHz experiments are more pronounced. Also, the differences between the CoV from 2.25 MHz and 5MHz
Figure 2.11: Simulated Definity™ microbubble results (point statistic, N=100, T=5) using a 75-kPa, 5-wavelength pulse: (a) Mean echo intensity vs. concentration, (b) coefficient of variation vs. concentration.
Figure 2.12: Simulated Definity\textsuperscript{TM} microbubble results (point statistic, N=20,000, T=1) using a 2.25MHz transducer, 75-kPa, 5-wavelength pulse: (a) Mean echo intensity vs. concentration, (b) coefficient of variation vs. concentration.

experiments are not significant, although 2.25 MHz CoV results are slightly depressed.

The model was also used to simulate significantly more independent backscatter measurements (N=20,000) and the mean echo intensity and CoV for this dataset is presented in Figure 2.12. The increased number of samples used in deriving the statistical metrics shows more clearly the linear relationship between microbubble concentration and mean echo intensity, and the inverse relationship between concentration and CoV. Figure 2.13 shows both low- (N=100) and high-sample (N=20,000) CoV curves for comparison. For higher concentrations, it appears a low number of samples (N=100) continues the trend established by the use of higher number of samples (N=20,000), and may be adequate to capture the statistical properties of the backscatter measurement in this concentration regime.

2.5.4 Windowed Time-Average Statistical Measures

Spatial averaging is considered by selecting a window of data, centred about the acoustic focus. The window size is then increased and an estimate of the mean echo intensity and CoV is determined based on the windowed time-averaged intensity.
Chapter 2. A Statistical Analysis of a Microbubble Pop. Response

Figure 2.13: Simulated Definity$^T$M microbubble results presented as coefficient of variation vs. concentration for low sample (N=100) and high sample (N=20000) datasets.

Figure 2.14 shows the impact of spatial averaging on Definity$^T$M by plotting window size against time-window averaged mean echo intensity measurements. It appears that the sparsity of microbubbles at the lowest concentrations result in a greater sensitivity to averaging. An overall absence of signal for 100 bubbles/ml is responsible for a decreased mean echo intensity up to $2\lambda$-sized window. Between 1 to 2 correlation lengths (1 correlation length = 5 $\lambda$), the window average changes significantly, particularly for low concentrations.

Figure 2.15 however demonstrates that averaging over a sufficient number of independent measurements is necessary.

Figure 2.16 show the impact on spatial averaging to the relationship between concentration with the mean echo intensity and CoV using select window sizes. Up to 2 correlation lengths, each concentration monotonically decreases in CoV.

2.5.5 Noise

Figure 2.17a plots the CoV for a typical experimental background measurement against simulated Gaussian noise. This noise was generated assuming a mean and standard deviation of 1, and the Hilbert transform was applied. Here, a consistent mean CoV
Figure 2.14: Impact of window size on (a) mean echo intensity and (b) CoV by exciting Definity\textsuperscript{TM} using 2.25MHz, 75kPa, 5-wavelength excitation pulse, after simulating 100 independent measurements per concentration (N=100).
Figure 2.15: Impact of window size on (a) mean echo intensity and (b) CoV by exciting Definity\textsuperscript{TM} using 2.25MHz, 75kPa, 5-wavelength excitation pulse, after simulating 20,000 independent measurements per concentration (N=20,000).
Figure 2.16: The effects on experimental data of 5-wavelength spatial averaging of the intensity envelope for by exciting Definity\textsuperscript{TM} using 5MHz, 75kPa, 5-wavelength excitation pulse.

Figure 2.17: Experimental background noise is compared with simulated Gaussian noise, in terms of (a) CoV and (b) frequency spectra.
Figure 2.18: Definity\textsuperscript{TM} microbubble simulation results with the addition of Gaussian noise to each trace before post-processing in order to simulate the effects of electronic Gaussian noise. The standard deviation of the noise is varied and identifies the extent of the noise.

is observed, corresponding to approximately 1. The simulated Gaussian noise was then scaled to the same magnitude as the experimental data before the FFT is performed, and Figure 2.17b presents the corresponding frequency spectra for experimental and simulated noise.

Figure 2.18 displays the result of increasing additive Gaussian noise to a series of simulated measurements produced at differing concentrations. Noise is added to the RF signal, similar to the contribution by a pre-amplifier, after which the RF-signal is post-processed to arrive at each datum.

## 2.6 Discussion

A correspondence between experimental and simulation results suggests the computer model is sufficiently suited to simulate backscatter measurements of a microbubble population under various low-amplitude acoustic conditions. In the process of validating the computer model, a number of expected and unexpected observations have been made, and their implications will be discussed. The model has the potential to be used to clarify the relationship between acoustic and experimental parameters to the variability
of microbubble backscatter measurements.

2.6.1 Linearity

The linear relationship between concentration and mean echo intensity has been demonstrated for microbubbles, although at closer inspection, there is a departure from linearity at the lowest considered concentrations. A distinction should be made between the mean echo intensity, which is the average of an ensemble of statistically-independent measurements, and a single instance in an ensemble, which is not expected to be proportional to microbubble concentration, but rather the mean is found within the 95% confidence interval (CI). At lower concentrations, the CI is expected to widen, and more independent samples are required to maintain the same CI. Jacobsmeyer predicts these requirements for scattering that adheres to Rayleigh statistics (Jacobsmeyer, 2007).

Linearity is of utmost importance in US quantification as relative microbubble concentrations can be inferred from mean echo intensity, which can then be used to infer the relative blood volume. Originally, the expected linear relationship between the number of scatterers and mean echo intensity was derived by Rayleigh, as a consequence of his random walk model (Rayleigh, 1880). The Orgasol™ results depicted in Figure 2.10 is an example of this relationship. However, as will be shown during the CoV discussion, the majority of microbubble measurements made can be classified as non-Rayleigh. Even for a signal that is non-Rayleigh, Jakeman and Pusey show that mean echo intensity maintains a linear relationship with the number of scatterers, and by extension, a linear relationship with the scatterer concentration (Jakeman and Pusey, 1976). Therefore, deviation from linearity at low concentrations could be attributed to averaging over an insufficient number of independent measurements to arrive at the mean echo intensity. Further, the mean echo intensity may be the same order of magnitude as the contribution from electronic noise. Noise can then bias the mean echo intensity estimation, which is evident in the lowest concentrations of experimental results shown in Figure 2.9a. The
impact of the microbubble frequency-dependent response to the mean echo intensity is significant. Both experiment and simulation concur that Definity® microbubbles respond with a greater intensity to a 2.25MHz pseudo narrowband pulse as compared to a 5MHz pseudo narrowband pulse for the same microbubble concentration and transducer geometry. Figures 2.9a & 2.11a demonstrate this very clearly.

2.6.2 Variability

Experimental and simulated CoV as a function of microbubble concentration show good relative agreement. While using Definity® microbubbles, simulation results found in Figure 2.11b show that the CoV for a single intensity envelope datum located at the centre of the focus, the point statistic, approaches the Rayleigh limit of 1 for higher concentrations. This is the expected result for interference by a sufficient number of uniform-amplitude scatterers. The increase in concentration leads to an increase in the subset of strong scatterers that dominate the population response, and therefore satisfy conditions required by the random walk model which is the basis of Rayleigh statistics.

The increase in CoV with a decrease in microbubble concentration signals a departure from the Rayleigh limit. As the number of excited microbubbles decrease, a key assumption underlying Rayleigh statistics becomes weaker. In particular, the Central Limit Theorem, required to ensure Gaussian-distributed real and complex components of the resultant pressure amplitude, breaks down. It is interesting that at decreasing concentrations, where the backscatter signal of individual microbubbles do not interfere with other microbubbles, that Figure 2.12b shows a continuous, unbroken monotonic trend between CoV and microbubble concentration when enough samples are collected.

Definity® microbubbles also respond more variably to a 5MHz pseudo narrowband pulse as compared to a 2.25MHz pseudo narrowband pulse for the same microbubble concentration and transducer geometry. The experimental data shows this dependence more so than the simulation data when Figures 2.9b & 2.11b are compared. This fre-
quency dependence may be attributed to fewer microbubbles effectively contributing to the overall signal due to a smaller focal volume of the 5MHz transducer.

2.6.3 Effects of Windowed Time-Averaging

By defining a window that is as wide as twice the pulse length, the mean statistic is shown to yield improvements in the mean echo intensity estimate through a significant decrease in CoV as shown in Figure 2.15. This figure shows that the CoV tends to decrease with increasing window size, which is predictable given the theoretical results in §1.8.3. When the number of samples used in the statistical analysis is less than sufficient for an accurate mean echo intensity estimate, particularly in the case when lower microbubble concentrations were sampled at N=100, Figure 2.14 shows that the CoV does not improve in any predictable fashion. A CoV decrease can be attributed to the estimation of the mean echo intensity by averaging over multiple points of an ergodic process such as the intensity envelope. However, a ROI should be drawn about a volume that is sufficiently uniformly insonated. Otherwise, the selected portion of the intensity envelope cannot be considered to be an ergodic process as the mean echo intensity would be expected to change significantly due to the inhomogeneous beam intensity.

2.6.4 Effects of Noise

Regardless of the care and attention made to the experimental setup, noise can be expected to impact experimental measurements to some degree. From Figure 2.9b, an unexpected drop in CoV at the lowest concentrations is observed. This can be explained by observing that the majority of experimentally-obtained intensity envelopes at the lowest concentrations are at the same magnitude as the noise floor.

Given that Gaussian noise could be contributed to by the various electronic components in the signal pathway (see Figure 2.2), experimental background measurements were compared with simulated Gaussian noise. Figure 2.17 shows the CoV of a particular
series of background measurements.

To simulate the impact on statistical properties, Gaussian noise was added to traces for a single simulated experiment. As Figure 2.18 demonstrates, the increasing dominance of noise significantly influences the CoV at the lowest concentrations. If noise from the pre-amplifier is not prevalent, measurements can be sensitive to weakly scattering individual microbubbles beyond the margins of the focal volume, as well as the prolonged damped oscillations of strongly scattering bubbles. Therefore, if the dynamic range of a detector is not a limitation, then even at the ultra-low concentrations considered, it is very unlikely that a point along the A-line would be a zero value, let alone consistently zero over an ensemble of statistically-independent measurements. If the concentration is so sparse that the number of microbubbles per resolution cell is $<< 1$, it should not be unsurprising to find a large CoV at low concentrations. On average, the CoV monotonically increases as the concentration approaches zero. However, due to the large mean error evident in Figure 2.9b, it is not surprising that for a single experiment corresponding to the data presented without noise in Figure 2.18, the CoV will not necessarily be monotonic.

### 2.6.5 Differences with *In-vivo* Conditions

There are at least 4 important distinctions between the environment microbubbles were placed in experiment and *in vivo*, and the relevance of the conclusions to the clinical context will be addressed.

Firstly, the microbubble resonance frequency is significantly influenced by microbubble proximity to capillary walls. See §1.7.3. A decrease in resonance frequency corresponds to a significant impact to the overall microbubble population frequency-dependent response. However, the fact that a different subpopulation of microbubbles will respond more favourably to one excitation frequency over another does not alter the general observations made regarding the statistical relationship between microbubble concentration
and backscatter intensity. However, these changes could have some bearing on the statistical relationship between excitation frequency and backscatter intensity for Definity™.

Ambient pressure also has an impact on resonance frequency. In experiment, the ambient pressure was relatively constant, primarily influenced by the height of the dilute microbubble reservoir used. However, microbubbles within the circulatory system can be exposed to different pressures. Higher pressure can increase the rate of diffusion and therefore affect the size distribution of the microbubbles.

In addition to differences in wall proximity and ambient pressure, the spatial distribution of microbubbles in vivo are more constrained. Microbubbles are limited to move through the microvasculature while in a flow cell, their position is not constrained within the sample volume limits. However, given the sparsity of the microbubble concentrations considered, microbubbles in an in vivo environment could have positions that are not correlated from one time frame to the next. Also, capillaries are small enough that multiple capillaries can be located within an imaging voxel. Therefore, the conclusions can be applicable as the presence of low concentrations of microbubbles in vivo can result in fully decorrelated microbubble positions from one backscatter acquisition to the next.

Lastly, whereas the bandwidth about the fundamental harmonic frequency of the backscatter signal is analyzed in this thesis, tissue clutter from similar B-mode, in vivo, US scans make it necessary to employ tissue-suppression schemes. Such tissue-suppression schemes typically send a series of excitation pulses designed to exploit the nonlinear backscatter signal from microbubbles, and subsequent backscatter measurements can be used to remove the linear tissue signal. The analysis of the bandwidth about sub- or superharmonic frequencies is typical in clinical and preclinical contrast studies. Although the microbubble backscatter is expected to be nonlinear with excitation pressure, results show that backscatter intensity is proportional to microbubble concentration. If backscatter signals are scaled, added, or subtracted, Rayleigh statistics predicts the resulting intensity is still proportional to scatterer concentration. In
addition, using a phasor formulation, it can be shown that Rayleigh statistics hold for the amplitude of each frequency component of a backscatter signal (Cobbold, 2007). Through experiment and simulation, nonlinear microbubble behaviour was observed and it has now been shown that the intensity for fundamental harmonic bandwidth-limited signal is proportional to microbubble concentration. It would be expected that a sub- or superharmonic bandwidth-limited signal would similarly be proportional to microbubble concentration. However, it is possible for the relationship between CoV and microbubble concentration to vary when analyzing other harmonics. For example, the impact of electronic noise can be a more prominent factor at higher harmonics due to their decreased amplitudes. Backscatter frequency-dependence may also change depending on differences in harmonic components of the backscatter signal for different subpopulations of microbubbles.

2.7 Conclusions

A computer model was created to simulate the overall backscatter echo of a bubble population within a finite volume under low acoustic amplitudes. This model took into account the nonlinear behaviour of individual encapsulated microbubbles, the population size distribution, the bubble spatial distribution, the transducer geometry, the transducer electromechanical impulse response, and the transmit pulse characteristics. Through the preceding analysis, the model has demonstrated that it is robust enough to yield statistical metrics that share the same frequency- and concentration-dependent characteristics as experimental measurements. The results produced through experiment and simulation have been used to build a high level of confidence in the following conclusions:

- mean microbubble echo intensity is proportional to microbubble concentration even at low concentrations
This thesis considered microbubble concentrations similar to in-vivo concentrations. A sufficiently high microbubble concentration regime was explored such that Rayleigh statistics was evident in measurements. This is equivalent to the speckle found in images of microbubbles in large blood vessels and highly perfused tissues such as the liver. Concentrations were subsequently decreased until the microbubble concentration was sufficiently sparse such that single bubble events could be witnessed. It was clear that measurement statistics significantly deviated from Rayleigh statistics at low concentrations. However, at these low microbubble concentrations it was shown that if a sufficient number of independent measurements were made, the mean echo intensity remained proportional to microbubble concentration.

- model and experiment show that CoV increases with decreasing microbubble concentration

The relationship between variability of microbubble concentration and backscatter measurements has been clarified. Using the CoV to quantify measurement variability, there is a reciprocal relationship between variability and bubble concentration. Measurements corresponding to high microbubble concentrations are expected to have a CoV of 1, the Rayleigh limit, while for lower concentrations, there is a departure from the Rayleigh limit, as the CoV increases nonlinearly.

However, at very low concentrations, background noise can negatively bias the mean echo intensity and variability of the signal. This is particularly prominent when the signal exceeds the background noise but is within an order of magnitude. Therefore, a signal close to the noise floor could result in a less variable measurement, however its mean echo intensity is significantly biased and no longer indicative of the microbubble concentration.

- CoV increases with increasing frequency, and decreases with increasing window size these parameters can be used to optimize clinical microbubble measurements
The nonlinear microbubble frequency dependence has been shown to effect measurement variability. Both the selection of the transducer and the centre-frequency of the pulse used to excite the transducer can modulate these results. In the case of the 2.25MHz transducer, both the total backscattering cross-section and sample volume were higher compared to the use of the 5MHz transducer for both experiment and simulation. Therefore a greater frequency-dependent microbubble response decreases measurement variability. Also, a larger sample volume defined by the transducer field will decrease measurement variability.

Beyond transducer and contrast agent selection, an appropriate measurement-processing methodology can help reduce measurement variability. Under ideal circumstances, a time-window corresponding to a volume of uniform microbubble perfusion and pressure can be selected. Such a time segment would be considered an ergodic process, and averaging over the window would reduce measurement variability. In the case of a non-uniform pressure field, such as that produced by a spherical transducer, it has been shown that averaging over a time-window corresponding in length to 2 pulse lengths is also an effective means of reducing measurement variability.
Chapter 3

Summary and Future Work

3.1 Summary

The conclusions represented in this thesis provide valuable insight into the relationship between US backscatter measurement variability, intensity, and microbubble concentration.

A computer model was created to simulate the backscattered signal of a population of microbubbles. This model accurately represents the time-varying field produced by a single-element transducer, models microbubble behaviour using a viscoelastic model, and takes into account the random spatial and size distribution of microbubbles. Simulated results were validated with experiment.

This thesis explored a microbubble concentration regime where, for the upper limit, microbubbles could be considered Rayleigh scatterers, and at lower concentrations microbubble echoes were recorded as discrete events and would not be expected to obey Rayleigh statistics. In the absence of noise, it was shown that the mean backscatter signal intensity was linear with microbubble concentration throughout this range. The CoV, on the other hand, significantly increased with decreasing microbubble concentration. Although linearity between mean echo intensity and concentration is an expected prop-

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property of Rayleigh statistics, the CoV was significantly greater than 1 for low microbubble concentrations. When electronic noise was considered, the mean echo intensity of the signal approached the noise floor, potentially biasing both mean echo intensity and variability at low concentrations. As hypothesized, microbubbles do not behave like Rayleigh scatterers at lower concentrations.

The centre-frequency of the excitation pulse (i.e. excitation frequency) was also shown to modulate measurement variability. As the excitation frequency decreased, the focal volume increased, exciting more microbubbles situated a particular distance from the transducer as a result. In addition, bubble resonance for different sizes of microbubbles were elicited based on the excitation frequency. Evidence correlated with the notion that a particular subpopulation of such microbubbles scattered more strongly than other bubbles at a specific frequency, effectively reducing the bubble concentration to that of the subpopulation. In light of this frequency-dependence, an appropriate selection of a number of transducer and microbubble properties can decrease measurement variability. Therefore, as hypothesized, there was shown to be a frequency and diffraction dependency of the backscatter response.

Lastly, experimental design and measurement-processing can help reduce variability. In accordance with the Central Limit Theorem, acquiring measurements at a sufficiently low pulse repetition frequency will realize different microbubble spatial distributions, making measurements statistically independent. This can justify the averaging of multiple measurements to estimate the mean echo intensity with reduced variability. In addition, if time points of an A-line correspond to voxels in the sample volume with a uniform beam amplitude, then the measurement is deemed ergodic and averaging measurements across these time points can also reduce measurement variability. It has been shown that if the number of independent samples is sufficient, then averaging over a window size limited to within 2-pulse lengths prior to averaging over the set of independent measurements can further reduce variability while maintaining an accurate estimate of
Chapter 3. Summary and Future Work

the mean echo intensity.

3.2 Outline of Future Work

The Rayleigh model is applicable when a sufficient number of scatterers are present and they are scattering with a uniform amplitude. However, this is not the case at low microbubble concentrations and where resonance exaggerates signals from bubbles within the focal volume. The K-distribution statistical model was first proposed by Jakeman and Pusey (Jakeman and Pusey [1976]) to address similar backscattering issues in radar. A review of the consequences to mean echo intensity and CoV by a K-distributed US backscatter signal are explored in this final chapter.

In the process of investigating the relationship between mean echo intensity, CoV, and microbubble concentration, a unified theory incorporating the K-distribution has been developed to account for differences between contrast agents and transducer characteristics. A power relationship will be proposed. Results from simulations will be shown below, however this theory remains to be verified experimentally. Nevertheless, in the future, a general relationship between key statistical parameters and experimental parameters is proposed to frame future hypotheses.

To further develop a thorough understanding of the statistical characteristics of contrast measurements made using clinical scanners, a broad range of contrast imaging schemes should be considered. This thesis focused specifically on one particular US imaging modality, namely fundamental harmonic imaging. However, a significantly greater understanding can be gained through further application of the computer model to simulate the results of multi-sequence pulses. This process is aided by the fact that the development of a model to understand backscatter measurement statistical properties has resulted in the creation of a general-purpose US backscatter simulation.
3.3 Modelling Non-Uniform Amplitude Ultrasound Scattering from Microbubbles Using the K-Distribution Model

The Rayleigh statistical framework assumes that a sufficient number of scatterers are present within a given voxel. If this assumption breaks down, then the result of the interference of the incoming microbubble backscatter signals, as defined in §1.8.1, no longer has Gaussian-distributed real and complex components. Hence, the Rayleigh statistical model no longer applies. A similar problem arises if a population of scatterers scatter non-uniformly, whereby only a proportion of scatterers make any significant contribution to the resultant signal. Not surprisingly, this problem has been tackled in other fields of research. In 1976, Jakeman and Pusey described a scenario of sea clutter signals in radar applications that have the same statistical properties as the linear scatterers considered for US (Jakeman and Pusey, 1976; Jakeman, 1980). To address the non-uniform amplitude scattering inherent in reflections from the variable surface of the sea, they described a new PDF that represents a more general form of the Rayleigh distribution, the K-distribution:

\[
PDF(A) = \frac{2b}{\Gamma(1 + \nu)} \left( \frac{bA}{2} \right)^{\nu+1} K_{\nu} (bA)
\]  

(3.1)

where \(A\) is the resultant amplitude of the backscatter interference, \(\Gamma()\) is the gamma function, \(K_{\nu}\) is the Modified Bessel function of the 2nd kind with order \(\nu\), and \(b\) and \(\nu\) are shape parameters. Jakeman and Pusey found that this form satisfied key requirements for representing a PDF for the radar cross-section. Because radar involves similar post-processing to US, the statistical models developed for the backscatter interference of radar scatterers may be applicable to the backscatter interference of US scatterers. Jakeman and Pusey derived the corresponding PDF of a single instance in time of the intensity
envelope, $I$:

$$PDF_N(I) = \frac{b}{\Gamma(M)\sqrt{I}} \left( \frac{b\sqrt{I}}{2} \right)^M K_{M-1} \left( b\sqrt{I} \right)$$ \hspace{1cm} (3.2)

where

$$M = N (1 + \nu)$$ \hspace{1cm} (3.3)

where $N$ is the number of scatterers contributing to the signal, and $M$ is considered to be the effective number of scatterers. They concluded that the 1st moment of the intensity PDF, the mean echo intensity, is similar to that of the exponential PDF:

$$\langle I \rangle = N \langle a^2 \rangle$$ \hspace{1cm} (3.4)

Although higher order moments of the intensity PDF are quite different, as $N \rightarrow \infty$, the higher order moments approach that of the exponential PDF (Jakeman and Pusey, 1976):

$$\langle I^n \rangle = \left( \frac{2}{b} \right)^2 n! \frac{\Gamma(n + M)}{\Gamma(M)}$$ \hspace{1cm} (3.5)

Therefore the CoV can also be defined for the K-distribution to be:

$$CoV = \sqrt{\frac{\langle I^2 \rangle}{\langle I \rangle^2} - 1} = \sqrt{1 + \frac{2}{M}}$$ \hspace{1cm} (3.6)

However, if all scatterers scatter uniformly, then Equation 3.6 can be simplified as a monotonically decreasing CoV with respect to increasing concentration, approaching a plateau of unity CoV for sufficiently high concentrations, similar to the trend observed in experiment:

$$CoV = \sqrt{1 + \frac{1}{N}}$$ \hspace{1cm} (3.7)

Future work can potentially show whether microbubble measurements do in fact adhere to K-distribution statistics for sparse microbubble concentrations.
3.4 Proposing a General Relationship Applicable to Various Backscatter Measurements at Low Scatterer Concentrations

A number of parameters change with excitation frequency, including resolution cell volume, $V$, the total backscatter cross-section, $\sigma_T$, and the percentage of the microbubbles that contribute to the bulk of the measured signal, $\chi$. To investigate the influence of these parameters, simulated results will be rescaled according to Equation 3.8:

$$Concentration : x \rightarrow Vx$$
$$MeanEchoIntensity : I \rightarrow \frac{I}{\sigma_T}$$
$$CoV : CoV \rightarrow \sqrt{\chi}CoV$$

The total backscattering cross-section is the summation over all diameters of the calculated backscatter cross-section at a specific Definity$^TM$ microbubble diameter times the number of microbubbles counted by the Coulter Counter$^TM$. The percentage of dominant microbubbles is calculated as the weighted average number of microbubbles based on the size distribution and backscatter cross-section of Definity$^TM$ microbubbles. Lastly, the resolution cell volume is considered to be a portion of the focal volume, approximated as a pill-box shape, depicted in Figure 1.3 with a diameter equal to the full-width-half-maximum (FWHM) of the focal volume of an acoustic field, and a thickness corresponding to the length of the pulse, specifically equal to 5 wavelengths. Note that the FWHM and pulse length vary as a function of excitation frequency.

3.4.1 Simulated Results

Figure 3.1 presents the scaled results as per Equation 3.8. Microbubble concentration is scaled by flow cell volume and resolution cell volume to arrive at the number of scatterers per resolution cell volume. In addition, simulated mean echo intensity and CoV
Figure 3.1: The effect of scaling bubble concentration to the number of scatterers per resolution cell volume, as well as mean echo intensity with total backscatter cross-section, and CoV with the percentage of dominant scatterers, on Definity\textsuperscript{TM} (DEF) and free gas bubble (FGB) simulation results: (a) Mean echo intensity vs. concentration, (b) CoV vs. concentration, (c) scaled mean echo intensity vs. # of scatterers per resolution cell volume, (d) scaled CoV vs. # of scatterers per resolution cell volume.

Figure 3.1 is scaled using the total backscatter cross-section and percentage of dominant scatterers, respectively. The total backscatter cross-section used is based on the results plotted in Figure 2.7b. For comparison, equivalent data for simulated free gas bubbles with the same position and size distribution as the simulated Definity\textsuperscript{TM} bubbles are presented.

Results from simulated Definity\textsuperscript{TM} microbubbles (DEF) and free gas bubbles (FGB) differ markedly in Figures 3.1a & 3.1b. However, these differences are eliminated by compensating for the total backscatter cross-section, resolution cell volume, and per-
percentage of bubbles that dominate the signal, as shown in Figures 3.1c & 3.1d, indicate that these parameters impact significantly the intensity and variability of backscatter measurements.

### 3.4.2 Discussion

The relative success in normalizing mean echo intensity measurements with respect to total backscatter cross-section is expected. Given the same size distribution and spatial distribution of microbubbles, the measured backscatter signal is expected to be proportional to the total backscatter cross-section. However, the manner in which mean echo intensity is dependent on frequency in backscatter measurements requires discussion. As can be appreciated from Figure 2.7, the total backscatter cross-section is nonlinearly-dependent on excitation frequency. The relative differences between mean echo intensity measurements at different frequencies and between Definity\textsuperscript{TM} and FGB's can be predicted from this numerically-derived theoretical total backscatter cross-section plot.

Mean echo intensity is not only dependent on the frequency-dependent total backscatter cross-section, but also the frequency-dependent resolution cell volume. It is not surprising that the resolution cell volume plays an important role in mean echo intensity as its size will proportionally increase the number of scatterers that are excited with a sufficiently high incident pressure. Changes in transducer geometry are also expected to influence mean echo intensity, as changes in the FWHM of the focal volume will alter the resolution cell volume accordingly.

To summarize, mean echo intensity is expected to be proportional to experimental parameters such as concentration, pulse length, and insonation amplitude, proportional to the square of the focal volume FWHM, and to vary nonlinearly with excitation frequency with lower mean echo intensity expected at higher frequencies. These expected results, if verified experimentally, will provide further validation that the simulation reflects the physics of typical backscatter measurements. Equation 3.9 summarizes the
Chapter 3. Summary and Future Work

The proposed relationship between mean echo intensity with total backscatter cross-section, $\sigma_T$, microbubble concentration, $x$, and resolution cell volume, $V$:

$$\frac{I}{\sigma_T} \propto Vx \rightarrow I \propto \sigma_T Vx \quad (3.9)$$

The same methodology is applied to understand the relationship between CoV and experimental parameters. Microbubble concentrations were considered such that proximity to the Rayleigh statistical characteristic of CoV of 1 was only observed for the highest concentrations. However, a value of 1 can no longer be considered an indicator of Rayleigh statistics for scaled CoV. Figure 3.1d shows that when the CoV is not close to 1, there is an approximate inverse cubic relationship between the number of scatterers per resolution cell volume and the scaled CoV.

Figure 3.1d was produced by scaling CoV by the square root of the percentage of dominant scatterers. The coincidence of the resulting data using this scaling factor demonstrates that CoV is also inversely proportional to the square root of the percentage of dominant scatterers within the overall population. This highlights the significance of a subpopulation of dominant scatterers over the overall number of microbubbles. The concept that a subpopulation of scatterers drives the statistical properties of a backscatter measurement was the basis for Jakeman and Pusey’s investigation into the use of the K-distribution model. In the case of a monodispersed population, then 100% of scatterers participate equally, regardless of the frequency or nature of the scatterers. The percentage of dominant microbubbles also accounts for the frequency-dependence of CoV as well as the differences encountered from different size distributions and types of scatterers.

In summary, from simulation results at low microbubble concentrations, the CoV is found to be approximately inversely proportional to the cubic root of scatterer concentration, pulse length, and the cubic root of the square of the focal volume FWHM, as well as inversely proportional to the square root of the percentage of dominant scatterers. Equation 3.10 summarizes the proposed relationship between CoV with the percentage
of dominant scatterers, \( \chi \), microbubble concentration, \( x \), and resolution cell volume, \( V \):

\[
\sqrt{\chi}CoV \propto \frac{1}{\sqrt[3]{Vx}} \rightarrow CoV \propto \frac{1}{\sqrt[3]{\chi \sqrt[3]{V} \sqrt[3]{x}}}
\]  

(3.10)

### 3.4.3 Further Generalizations

To understand the nature of the approximate cube-root relationship between CoV and microbubble concentration, the K-distribution scattering model was considered further. The K-distribution model can account for non-uniform scattering as well as sparse scattering. However, if uniform scattering is assumed amongst the dominant scatterers, then the relationship between number of scatterers per resolution cell volume and CoV is described by Equation 3.7. This equation is plotted in Figure 3.2. Equation 3.7 is modeled as \( CoV \propto N^C \) where \( C \) is a power-fit parameter and \( N \) represents the number of scatterers. Comparing with Equation 3.10 as \( N \rightarrow 0 \), results in \( C \rightarrow -\frac{1}{3} \). It is also clear from Figure 3.2 that for \( N > 10 \), CoV approaches 1, consistent with the Rayleigh model.

To understand the discrepancy between the approximate cubic root relationship observed in Figure 3.1d and the theoretical square root relationship, a simplified simulation was performed. No attenuation, impulse response, electromechanical response, or modulating scatterer response were considered. Each scatterer would scatter the incident waveform exactly. Multiple cases were considered including where: (1) scatterers were distributed uniformly along the axial centreline, (2) along a line angled at 45°, (3) as well as uniformly across a plane angled at 45°. The resolution cell volume was defined as the length of the rectangular excitation pulse. Figure 3.2 present the results against the expected K-distribution relationship. The resulting curve fits in Figure 3.3 show that the square root relationship does in fact hold for \( 0.01 < N < 0.1 \). However, as the instantaneous slope of the N-CoV graph modulates from 0.5 to 0 over \( 0.1 < N < 1 \), an average slope that approximates \( \frac{1}{3} \) can be observed. It appears that the 3D spatial distribution based on the 45°-oriented flow cell results in a decrease in the average slope as incoming
individual scattered signals do not arrive with a uniform temporal distribution.

The significance of the percentage of dominant scatterers to the CoV should not be underestimated. Equation 3.10 can be rewritten by generalizing the cubic root as $\gamma$, and defining the number of scatterers in a resolution cell volume to be $V_x$, or $N_{tot}$, and the effective number of dominant scatterers to be $\chi V_x$, or $N_{dom}$. If $\gamma$ is substituted with the experimentally ascertained power, $\frac{1}{3}$, the CoV becomes:

$$CoV = \left( \left( \frac{V_x}{(\chi V_x)^{\frac{1}{\gamma}}} \right)^{\frac{1}{\gamma}} \right) = \left( \frac{V_x}{(\chi V_x)^{3}} \right)^{\frac{1}{3}} = \left( \frac{N_{tot}}{N_{dom}^{3}} \right)^{\frac{1}{3}} \quad (3.11)$$

Equation 3.11 highlights the greater impact from increasing the proportion of dominant scatterers within a population of microbubbles over increasing the overall number of microbubbles. The inverse square root dependency can be explained in a similar manner to the dependence of CoV on total number of microbubbles. Due to the extremely small number of microbubbles that dominate the signal (< .5% for Definity$^{TM}$), the effective number of dominant scatterers will fall within a similar range depicted in Figure 3.3a. However, it is conceivable that the effective number of dominant scatterers could be significantly greater, therefore moving along the curve depicted in Figure 3.2. Therefore,
Figure 3.3: CoV as a function of # of scatterers per resolution cell volume as per the simple simulation and K-distribution model for (a) # of scatterers per resolution cell volume ranging between 0.01-0.1, and (b) 0.1-1.

the power relationship of the effective number of dominant scatterers is a function of itself, and can be related to CoV in general using the exponent $\delta$,

$$CoV = \left( \frac{(Vx)^{\frac{1-\gamma}{\gamma}}}{{(\chi Vx)^{\frac{1}{\gamma}}}} \right)^{\gamma \delta}$$  \hspace{1cm} (3.12)

Therefore, if the effective number of dominant scatterers fall within a range depicted in Figure [3.3b] resulting in $\gamma = \frac{1}{3}$ and $\delta = \frac{1}{3}$, the total number of scatterers becomes inconsequential to the CoV and highly reliant on the effective number of dominant scatterers:

$$CoV = \left( \frac{(Vx)^{0}}{(\chi Vx)^{\frac{1}{3}}} \right)^{\frac{1}{3}} = \left( \frac{1}{N_{dom}^3} \right)^{\frac{1}{3}} = \frac{1}{N_{dom}^3}$$ \hspace{1cm} (3.13)

or in the event that there is an abundance of microbubbles, but only a small subpopulation dominate the signal, then $\gamma = 1$ and $\delta = \frac{1}{3}$ resulting in:

$$CoV = \left( \frac{(Vx)^{-2}}{(\chi Vx)^{\frac{1}{3}}} \right)^{\frac{1}{3}} = \left( \frac{1}{N_{tot}^3 \chi} \right)^{\frac{1}{3}} = \frac{1}{N_{tot}^3 \chi^{\frac{1}{3}}} = \frac{1}{N_{tot}^3 N_{dom}^3}$$ \hspace{1cm} (3.14)

Summarizing, the simulation has helped lead to a potential relationship proposing that mean echo intensity is expected to be proportional to total backscatter cross-section, resolution cell volume and microbubble concentration. As a consequence, mean echo
intensity would also be proportional to pulse length and insonation amplitude, and pro-
portional to the cubic root of the square of the focal volume FWHM. The nonlinear
relationship between mean echo intensity and excitation pulse centre-frequency can be
predicted by calculating nonlinear frequency-dependent total backscatter cross-section.

Therefore the CoV is proposed to be inversely proportional to a fractional power,
\( \gamma \), ranging from 0-0.5 of the resolution cell volume and concentration, while being in-
versely proportional to a similar fractional power, \( \delta \), of the percentage of the effective
number of scatterers that dominate the overall backscatter signal. For the extremely
low bubble concentrations considered, the simulation yielded results where \( \gamma \) and \( \delta \) were
approximately \( \frac{1}{3} \) and \( \frac{1}{2} \), respectively. As a consequence, CoV is thought to be inversely
proportional to the cubic root of the pulse length and the square of the focal volume
FWHM. The nonlinear relationship between CoV and excitation pulse centre-frequency
can be predicted by calculating the weighted average number of scatterers that contribute
to the summation of the total backscatter cross-section.

### 3.5 Further Applications of the Model to other US

#### Imaging Modalities

At the heart of the model is the ability to capture the bubble population response by
simulating the behaviour of individual microbubbles. The model has been validated
by using the fundamental harmonic imaging scheme. Therefore, it is conceivable that
the model can be used to simulate a variety of different US imaging conditions, and
accommodate additional layers of complexity.

#### 3.5.1 QCEUS using Contrast Imaging Schemes

This thesis presents convincing experimental and numerical evidence of the linear rela-
tionship between intensity and concentration of microbubbles under an excitation and
measurement protocol characteristic of fundamental harmonic imaging. However, the majority of clinical measurements utilize various contrast imaging schemes in order to remove tissue signal effectively. Such schemes include Pulse Inversion (PI), Amplitude Modulation (AM), as well as their various combinations. A similar experimental and numerical validation using these various imaging schemes would be useful to affirm that the linear relationship between intensity and concentration exists. As well, such work could potentially highlight which contrast imaging scheme produces the least variable measurements based on the uniformity of individual microbubble responses.

Once validated across existing imaging schemes, the model can also be used to predict new and better contrast imaging schemes, tailored to the acoustic properties and size distribution of a particular microbubble contrast agent. Through comparison, such simulations can also help to justify why clinicians find that some schemes are more superior to others.

### 3.5.2 Ordered vs. Disordered Vascular Trees

A uniformly random spatial distribution of microbubbles has been assumed throughout this thesis. An argument was made that a uniform spatial distribution could be encountered in vivo where the vasculature is sufficiently dense. However, it has been recognized that vascular organization plays a crucial role in blood flow dynamics [Karshafian et al., 2003] and may lead to departures from a uniform spatial distribution of microbubbles in various regions of the body, like the kidney for example. The simulation can be sufficiently modified to accommodate the correlated position of microbubbles as they travel through a virtual network of ordered as well as disordered vascular trees.

The model can be used to simulate various local dynamic dose administrations within the context of an ordered or disordered vasculature. The parameters derived from a simulated bolus or negative bolus (i.e. disruption-replenishment) can be assessed for their accurate and precise representation of blood volume and flow parameters. The
effect of filling on the overall signal statistics can be determined.

### 3.5.3 Improving the Physical Complexity

The use of more advanced nonlinear differential equations to describe the radial changes of a bubble will help capture subtle microbubble behaviour. Increasing the sample volume will require more solving more bubble-dynamics equations, but be more representative of experimental conditions. Lastly, predicting attenuation due to the presence of microbubbles will also help simulate a more realistic backscatter measurement, particularly for high concentration cases.

Presently, the microbubble is modeled as a viscoelastic body. This behaviour is acceptable for low acoustic conditions. However, the implementation of the Marmottant model should preserve the solution at low acoustic amplitudes while capturing bubble behaviour such as the compression-only phenomenon observed at higher acoustic amplitudes.

Due to memory constraints, the number of microbubbles and therefore the sample volume size was limited in this thesis to the dimensions approximate to the largest focal volume considered. The flow cell constrained the microbubbles to a 2cm thick rectangular volume. However, simulating clinical scan planes will require a significantly larger sample volume. The power and flexibility of the computer simulation is dependent on the system memory and the memory and the number of cores of the CUDA-supported NVIDIA graphics card that are present. The serial algorithm implemented by Field II creates significant memory challenges for optimal performance. The implementation of a robust parallel algorithm to calculate individual impulse response to substitute for FieldIII would allow the sample volume to be considerably bigger. Using a graphics card with more ”compute cores” would allow more solutions to be calculated in parallel, and therefore would reduce the overall solution time.

Incorporating the effects of multiple scattering may be the most challenging improve-
ment to the model. This thesis has been limited to low-amplitude excitation not only to avoid the possibility and consideration of bubble cavitation, but also to constrain bubbles to the radial oscillation mode and to minimize multiple scattering effects. With the latter assumption, the solutions of multiple microbubbles can be solved simultaneously. However, if scattering from microbubbles are assumed to be significant enough to influence adjacent microbubble behaviour, then solutions must be calculated in a more complex and methodical manner.

One proposed methodology to address multiple scattering would be to solve for microbubble backscatter in order of distance from the transducer. On the basis of superposition, the original convolved excitation pulse and the radially-attenuated scattered waveform from adjacent previously-excited bubbles would be used as the excitation pulse for the next series of microbubbles. A table would help to keep track of which microbubbles are expected to influence other microbubbles. Of course, a similar calculation will be required for the back-propagation of the scattered pressure waveforms. Effectively, 2 solutions will be generated from conceivably every microbubble. Such a rigorous calculation would therefore take into account microbubble-associated attenuation and the effects of large amplitude excitations. Insight into thermal effects and therapeutic effects such as sonoporation could be derived.

3.6 Concluding Remarks

US contrast agents are currently being used in research hospitals to make qualitative and quantitative inferences of diseased tissues. This thesis tested a basic assumption that a linear relationship existed between microbubble concentration and US backscatter intensity. Findings confirmed this relationship holds for the case of fundamental harmonic imaging, albeit with conditions with respect to accuracy. The present work assessed the sensitivity of intensity measurements to the number of measurements taken,
microbubble shell parameters, US excitation frequency, US transducer geometry, and averaging-window size. By defining an appropriate scanning and analysis protocol, encountering low concentrations of microbubbles in clinical practice can be sure to yield useful information about disease progression.

As a matter of interest and further investigation, the physical basis of microbubble sparsity was considered. At very low concentrations, microbubbles were shown to be non-Rayleigh scatterers. By utilizing the numerical model developed in this thesis to predict backscatter from a microbubble population, a general relationship was postulated between key statistical parameters and experimental variables. If this relationship is validated, it can provide a direct means of evaluating the accuracy of US contrast measurements, and a tool for clinicians to help optimize their US contrast protocols.
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