MEASURING BLOOD PRESSURE USING MICROBUBBLES AND ULTRASOUND

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science
Graduate Department of Medical Biophysics
University of Toronto

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Abstract

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Blood pressure is one of the principal vital signs and an important disease indicator. It is known that hypertension leads to an increased risk of stroke, heart attack and chronic renal failure, while hypotension can lead to dizziness and fainting. Current non-invasive techniques used to monitor blood pressure (palpation, auscultation and oscillometry) can only measure the local pressure within the brachial artery and invasive methods such as catheterization are required when local pressure must be known elsewhere (such as within the chambers of the heart).

Gas microbubbles have a high compressibility, which make them very efficient sound scatterers. As another consequence of their high compressibility, microbubbles can be compressed by the pressure of the fluid around them, which affects their scattering properties. Due to recent progress in shelled ultrasound contrast agents and the development of almost monodispersed microbubbles, we believe it could now be possible to measure blood pressure using microbubbles as non-invasive manometers, an idea first suggested more than 30 years ago. In this thesis, both simulations and in vitro experiments will be used to investigate the changes related to the resonance of bubbles and how the concept of bubble size population affects the accuracy of this technique. In particular, it will be shown how shell dynamics dominates the response of microbubbles to blood pressure.
Dedication

For my family, Monique and Jean, who were always there to support me...
Acknowledgements

This work would not have been possible without the help of many people. First, I am very thankful to my supervisor, Peter N. Burns, for his precious support and mentorship, which has kept me on the right track during the full duration of my Master’s degree. I am also grateful to my supervising committee, Don Plewes and Richard Cobbold, for sharing their expertise with me. I would like to thank in addition Ross Williams, Athavan Sureshkumar, Brendan Lloyd, Emmanuel Cherin, David Goertz, Peter Bevan, Nikita Reznik, John Hudson, Kogee Leung and Minseok Seo, for giving me support in completing this project.

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<th>Abbreviation</th>
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<tbody>
<tr>
<td>CoV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>FWHM</td>
<td>Full-width half maximum</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>ODE</td>
<td>Ordinary differential equation</td>
</tr>
<tr>
<td>RMS</td>
<td>Root mean square</td>
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<tr>
<td>SNR</td>
<td>Signal-to-noise ratio</td>
</tr>
<tr>
<td>Symbol</td>
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<td>-----------------------------------------------------------------------------</td>
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<tr>
<td>$\alpha_p$</td>
<td>Free fundamental parameter for pipes</td>
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<tr>
<td>$\Gamma$</td>
<td>Gamma function</td>
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<tr>
<td>$\delta$</td>
<td>Total microbubble damping</td>
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<td>Shell damping</td>
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<td>$\delta_{\text{viscous}}$</td>
<td>Viscous damping</td>
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<td>$\delta(t - t_i)$</td>
<td>Delta function</td>
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<td>$\Delta P$</td>
<td>Pressure difference across pipe length</td>
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<td>$\epsilon'_{10}(\alpha)$</td>
<td>Womersley parameter 2</td>
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<td>$\zeta$</td>
<td>Shell surface tension second derivative</td>
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<td>$\eta$</td>
<td>Dynamic viscosity</td>
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<td>Dynamic viscosity of water</td>
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<td>$\kappa$</td>
<td>Polytropic gas constant</td>
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<td>$\rho$</td>
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<td>Mass density of bubble gas core</td>
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<td>Standard deviation of bubble radius size distribution</td>
</tr>
<tr>
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<td>Surface tension of water</td>
</tr>
<tr>
<td>$\phi$</td>
<td>Phase</td>
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</table>
\( \chi \) Shell elasticity
\( \psi \) Harmonic oscillator phase
\( \Psi \) Net phase of oscillator
\( \omega \) Angular frequency
\( \omega_o \) Angular natural resonance frequency
\( \omega_{res,P} \) Angular resonance frequency (pressure response)
\( \omega_{res,R} \) Angular resonance frequency (radial response)
\( A \) Area
\( A_i \) Amplitude of complex number
\( c \) Speed of sound
\( d \) Pipe diameter
\( f_o \) Natural resonance frequency
\( f_{res} \) Resonance frequency
\( g_c \) Centrifugal acceleration
\( H \) Transfer function assuming reciprocity (\( H = H_R = H_T \))
\( H_B \) Microbubble transfer function
\( H_R \) Transducer transfer function on receive
\( H_T \) Transducer transfer function on transmit
\( \vec{k} \) Wavevector
\( K_{liquid} \) Total kinetic energy in liquid
\( K_x \) Bessel function of second kind of order \( x \)
\( L \) Pipe length
\( m_\nu \) Moment \( \nu \) of probability distribution
\( M_{10}'(\alpha) \) Womersley parameter 1
\( N \) Total number of microbubbles
\( N_{AVE} \) Number of measurements compounded
\( p_g \) Gas pressure
\( p_{g,e} \) Gas pressure at equilibrium
\( p_l \) Pressure in the liquid at the boundary of the microbubble
\( p_v \) Vapour pressure
\( p_\infty \) Pressure at infinity
\( P \) Pressure
\( P(\chi) \) Probability distribution of variable \( \chi \)
\( P_{ac} \) Acoustic pressure [kPa]
\( P_{\text{threshold}} \) Threshold acoustic pressure for subharmonic stability [kPa]
\( P_o \) Hydrostatic pressure (most commonly blood pressure) [mmHg]
\( P_{\text{scatt}} \) Scattered pressure field [kPa]
\( P_{\text{input}} \) Input pressure pressure field [kPa]
\( Q \) Quality factor
\( Q_v(t) \) Volumetric flow
\( r \) Radial coordinate (spherical)
\( r_{\text{buck}} \) Buckling radius
\( r_o \) Observation point location
\( r_{\text{Elas1}} \) Elastic radius limit 1
\( r_{\text{Elas2}} \) Elastic radius limit 2
\( r_{\text{ruptured}} \) Rupture radius
\( r_p \) Pipe radius
\( \vec{R} \) Distance of bubble from observation point \( r_o \)
\( R(t) \) Microbubble instantaneous radius
\( R_o \) Microbubble radius at equilibrium
\( R_{eq} \) Equivalent pipe resistance
\( R_p \) Pipe resistance
<table>
<thead>
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<th>Symbol</th>
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<tbody>
<tr>
<td>$t$</td>
<td>Time</td>
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<tr>
<td>$\vec{v}$</td>
<td>Velocity field</td>
</tr>
<tr>
<td>$u_b$</td>
<td>Buoyancy velocity</td>
</tr>
<tr>
<td>$v_1, v_2$</td>
<td>Mean velocity within cross-sectional area</td>
</tr>
<tr>
<td>$V_{\text{calib}}$</td>
<td>Output voltage using Quartz plate reflector</td>
</tr>
<tr>
<td>$V_{\text{in}}$</td>
<td>Input voltage</td>
</tr>
<tr>
<td>$V_{\text{out}}$</td>
<td>Output voltage</td>
</tr>
<tr>
<td>$x$</td>
<td>Fractional change in the bubble radius from equilibrium</td>
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<td>Fourier transform of fractional change in the bubble radius from equilibrium</td>
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Chapter 1

Introduction

1.1 History, relevance and limitations of blood pressure measurements

While historical evidence suggests that the Egyptians, as early as 3150 BC, noticed blood pressure pulsation using simple palpation, the concept of blood pressure as we know it in modern medicine was first evoked by William Harvey who suggested in 1616 that the body contained a finite amount of blood. His idea initially faced skepticism since it questioned bloodletting practices, and the first experimental blood pressure measurements were made only in 1733, by inserting a brass pipe inside a horse’s crural artery. In 1828, Poiseuille published an equation for the laminar flow of blood in pipes relating vascular resistance and blood pressure, based on empirical observations, about 20 years before the Navier-Stokes equations were derived in their current form (1843-1845). These developments, combined with observations demonstrating correlation between anomalous blood pressure and pathologies, motivated the development of a portable non-invasive sphygmomanometer (from the Greek sphygmos for pulse). In 1855, Vierordt proposed to use the counter pressure (i.e., the pressure required to stop the arterial blood flow) to measure blood pressure, but his lever sphygmograph had little clinical application.
Chapter 1. Introduction

Etienne Marey (1860) and Samuel Siegfried Karl Ritter von Bash (1881) then devised a sphygmomanometer using unilateral pressure, a much more reliable procedure than Vierordt’s lever compression. The manometer using a cuff, as we know it today, was first introduced by Riva-Rocci in 1896. Initially the counter pressure obstruction was determined using manual palpation of the wrist, which was more or less accurate. The auscultatory method (i.e., combined use of a stethoscope and sphygmomanometer) was developed by Nikolai Korotkoff in 1905. Korotkoff observed that as the counter pressure of the cuff is lowered slightly more than the systolic pressure, the blood can only pass through the arm during systole in a turbulent way producing sound. As the counter-pressure reduces below the diastolic pressure, the sounds disappear. Korotkoff’s sounds are still used clinically to measure the brachial artery systolic/diastolic blood pressures.

Nowadays, the concept of blood pressure is central in medicine. Recent statistics suggest that 29% -31% [5, 6] of the United States population older than 18 years is affected by hypertension (corresponding to 58-64 million people). This number goes up to 50% for population older than 65 years. This has significant effects since high systemic blood pressure is often correlated to important short- and long-term risks. Hypertension is the most common risk factor for heart attack and stroke, and is an important contributor to heart failure, left ventricular hypertrophy, aneurism, chronic kidney disease as well as end-stage renal disease. Moreover, since the cardiovascular system is very complex, hypertension may be localized within a region of the vascular system, yielding a normal pressure elsewhere. For instance, an individual may suffer from portal vein hyper-pressure (a specific case of particular clinical importance in this thesis that will be discussed later) but present a perfectly healthy brachial artery blood pressure. At the moment, whenever pressure needs to be accurately known elsewhere than the brachial artery, invasive methods such as catheterization are employed (where a local micromanometer measures the blood pressure directly within the blood vessel through arterial lines). Catheterization, in addition to being expensive, involves higher risks for
the patient such as bleeding, thrombosis and infection. Such invasive methods are beneficial only for patients in intensive care, and a non-invasive method for measuring blood pressure in non-limb blood vessels has yet to be achieved.

### 1.2 Basics of the physics of blood circulation

A precise modelling of the motion of blood, a corpuscular fluid, within an organism, a non-symmetric and chaotic vascular bed, is no trivial task. It has been shown that simple physical concepts and assumptions can describe accurately the first order behaviour of blood circulation. From the perspective of fluid flow, blood can be regarded as an incompressible Newtonian fluid, i.e., a fluid which can be described fully by a scalar viscosity, whose exact value depends on the hematocrit. The Navier-stokes equation describes the flow properties of such fluids:

\[
\frac{\partial \vec{v}}{\partial t} + \vec{v} \cdot \nabla \vec{v} = -\frac{1}{\rho} (\nabla P + \eta \nabla^2 \vec{v}) \tag{1.1}
\]

where \( \vec{v} \) is the velocity, \( \rho \) is the density and \( \eta \) is the viscosity. The incompressibility of blood implies that \( \nabla \cdot \vec{v} = 0 \) or, using Gauss’ theorem, that the blood flux is conserved \( (v_1 A_1 = v_2 A_2 \text{ where } A \text{ is a cross-sectional area}) \) through the vascular system. Blood velocity through the different levels of the vascular system is shown in figure 1.1. Notice that the total area of capillaries is \( \sim 1000 \) higher than the cross-sectional area of the aorta, and therefore blood velocity in the aorta is much higher than in capillaries. Though, contrary to popular belief, blood velocities in the aorta and in the vena cava are roughly the same, since both have a similar net cross-section (refer to figure 1.1).

Although blood flow is intrinsically pulsatile, a steady state solution yields a rough estimation of the systemic behaviour (steady state assumptions are good in the venous system, but fail in arteries close to the heart). In the particular case of non-pulsatile flow in a long cylindrical pipe with length \( L \) and radius \( r_p \) (with the no slip boundary
Figure 1.1: Schematic view of the mean velocity of blood through the vascular system. The initial velocity in the aorta was calculated based on the initial pumping rate of the heart. Blood flux is constant [1].

condition), we recover the well known Poiseuille solution for the volume flow as shown in figure 1.2. The Poiseuille equation describes the resistive force that will act against a fluid moving in a pipe. As in an electric circuit, it makes sense to define the resistivity $R_p$ as $R_p Q_v = \Delta P$, which is $R_p = \frac{8nL}{\pi r^4_p}$ for a Poiseuille pipe. A direct consequence is that the resistance of blood vessels will considerably increase as the radius size decreases; for instance, the resistance of a 10 $\mu m$ capillary will be roughly $10^8$ times higher than the resistance of a 1 cm artery (though this doesn’t account for the corpuscular nature of blood, which affects considerably blood viscosity in small vessels). A thickening of the blood by increasing the hematocrit will also increase the vascular resistance through the viscosity. At high Reynolds number (if the inertia of the fluid becomes high), changes in pressure $\Delta P$ may arise through blood acceleration and deceleration. In very short pipes, nearly all of the impedance is attributable to blood acceleration (potential energy converted to kinetic energy).
Figure 1.2: Schematic view of Poiseuille flow through long pipes. The resistance is inversely proportional to the 4th power of the radius.

In intermediate length pipes, the pressure difference can be computed according to a viscosity corrected Bernoulli equation \[7\]:

\[
R_p = \frac{\rho \alpha_p}{8\pi \eta L} \frac{\Delta P}{\sqrt{1 + \frac{\alpha_p \rho^4}{32 \eta^2 L^4} \Delta P^2}}
\]

(1.2)

where \( \alpha_p \) is a free fundamental parameter related to the pipe geometry. This equation recovers the Poiseuille pipe resistance \( R_p = \frac{8\eta L}{\pi r_p^4} \) as \( L \to \infty \), converges toward \( R_p = \sqrt{\frac{\alpha_p \rho \Delta P}{\pi r_p}} \) as the pin-hole geometry is reached (when \( L \to 0 \)).

For pulsatile flow, the Poiseuille equation can be extended assuming linearity of the Fourier decomposition, Newtonian fluids and a rigid tube \[8, 9\]. The relation between volumetric flow and harmonic pressure gradient is then:

\[
Q_v(t) = \frac{8}{R_f} \frac{M'_{10}}{\alpha^2} \Delta P \sin(\omega t - \phi + \epsilon'_{10})
\]

\[
\alpha = r_p \sqrt{\frac{\rho}{\eta \omega}}
\]

\[
R_f = \frac{8\mu L}{\pi r_p^2}
\]

(1.3)

where \( M'_{10}(\alpha) \) and \( \epsilon'_{10}(\alpha) \) are the Womersley parameters \[8\].

A system of pipes will follow similar resistive laws to circuits due to the incompressibility of blood. Therefore the equivalent resistance of pipes in series is \( R_{eq} = \sum_i R_{p,i} \), while the resistance of pipes in parallel is \( \frac{1}{R_{eq}} = \sum_i \frac{1}{R_{p,i}} \). Yet the human cardiovascular
system is very complex and it is impossible, in a realistic setup, to predict the pressure difference between two distinct points within the body. For instance, the pressure within the brachial artery is not indicative of the pressure within the portal vein.

1.3 A Clinical case: blood pressure in portal system

1.3.1 Physiology of the disease

Portal vein hypertension is a common complication of liver cirrhosis, where the resistance of the portal-hepatic bed is drastically increased due to fibrosis. This causes an increase of the pressure within the portal vein above 12 mmHg, from 5-10 mmHg in healthy individuals. Other factors affecting the liver net vascular resistance such as portal/hepatic vein thrombosis and right heart failure, may also cause portal vein hyper-pressure. Literature suggests that the actual clinical consequences of portal vein hyper-pressure are independent of its cause. In addition to ascites, liver failure and splenomegaly, portal hypertension causes the dilation of portacaval anastomoses, in order to relax the portal vein pressure, leading to the formation of esophageal, gastric and anorectal varices (this happens in 90% of all cirrhotic patients) [10]. In particular, the esophageal and gastric varices may cause important variceal haemorrhage if ruptured (30% of all varices), imposing a constant life-threatening risk for the patient (30-50% mortality risk). Liver cirrhosis is a common disease in America, afflicting 2000 out of 100000 people [10].

1.3.2 Diagnosis of disease

Portal vein pressure cannot be measured through usual sphygmomanometry techniques. Although it is in theory possible to catheterize through the jugular vein and inferior veina cava, then puncture through the liver to access the portal circulation in order to measure the local blood pressure with a manometer, such a procedure is extremely invasive and involves high risk for the patient. For this reason, the gold standard for diagnosis of
portal hypertension relies solely on the symptoms (Child-Pugh score), and not directly on pressure values, making any early diagnosis of the disease very difficult. A non-invasive way to measure blood pressure (i.e., gauge pressure) with a pressure resolution of \( \sim 10 \) mmHg within non-limb vessels would have important value in the diagnosis of portal vein hyper-pressure.

The problem of non-invasively measuring blood pressure deep within the body has attracted considerable attention within the last 15 years. Beulen [11] showed that it is possible to extract changes of blood pressure (relative blood pressure) in arteries by measuring precisely their compliance to blood pulses using ultrasound. Yang [12], Urchuk [13] and Thompson [14] have shown that it is possible to measure pressure difference (relative pressure) based upon velocity profiles. More recently, Dharmakumar [15] proved through simulations that it could be possible to measure relative blood pressure by using microbubbles, coated with highly susceptible nanoparticles, with MRI. While both MRI and ultrasound can deliver the required precision for a relative dynamic measurement, these techniques can not deliver gauge pressure measurements as the results are expressed as differentials. It has been proposed that monitoring qualitatively the Doppler waveform within the hepatic vein can potentially indicate severe hypertension \( (> 15 \text{mmHg}) \) [16], although, the technique suffers from reproducibility issues [17]. A true method for measuring the gauge pressure within the portal vein has yet to be achieved.
1.4 Microbubbles as local non-invasive manometers

1.4.1 Non-linear scattering properties of microbubbles and applications

A recent trend in imaging is to use contrast agents in order to improve conventional imaging techniques. For example, parametric particles (e.g., iron oxide, gadolinium particles) can be used in magnetic resonance imaging due to their high magnetic susceptibility. In ultrasound, micro-gas spheres of high compressibility shelled by a thin coating, commonly referred to as microbubbles, are used. Microbubbles are smaller than red blood cells (1-10 \( \mu m \) in diameter), but big enough such that they cannot diffuse through the wall of blood vessels, making them intravascular agents (MRI contrast agents can leak outside of blood vessels). Blood is a poor acoustic scatterer of ultrasound and will appear black on a B-mode image relative to tissue. When microbubbles are injected, blood will brighten up on a B-mode image due to the coupling of the radial motions of the microbubbles’ walls with the surrounding liquid, considerably increasing the backscattered signal.

Moreover, microbubbles behave as non-linear scatterers even when driven at low acoustic pressure and will emit signal at integer (harmonic) and fractional (subharmonic, ultraharmonic) multiples of the insonication frequency (figure 1.3). On the other hand, tissues behave closely to linear scatterers with only a weak emission of harmonics. At even higher pressures, ultrasound causes microbubble destruction.

Many of the properties of microbubbles are already extensively used in clinical ultrasound. Contrast imaging (pulse-inversion, second harmonic, subharmonic, etc.) can create a map of vascular density by filtering away the fundamental of the scattered signal, which is emitted both by tissue and microbubbles, and keeping the nonlinear component which is emitted only by microbubbles. Such mapping become very pertinent, for instance, in liver cancer detection. More recently, it has been shown by Hudson et al. [18] that disruption-replenishment techniques, where bubbles in a region are disrupted using
Figure 1.3: At low excitation pressure (1 kPa), the microbubble responds almost linearly (second and third harmonics amplitudes, while present, are orders of magnitude smaller than fundamental) and scatters mostly at the fundamental frequency (left) when insonicated by a narrowband excitation. As acoustic pressure is increased (100 kPa), harmonics appear in the scattered spectrum (right).

high power pulses and local replenishment of the microbubbles is monitored through normal B-mode, yield quantitative information about the local vascular flow, blood vessel size distribution and fractal index. An example of contrast imaging of a renal tumour is shown in figure 1.4.

1.4.2 The susceptibility of microbubbles to blood pressure

As another consequence of their high compressibility, it has been suggested about 30 years ago by Fairbank et al. [19] that unshelled microbubble scattering properties are also affected by the static pressure and therefore could provide a direct way to measure blood pressure locally deep within the body using ultrasound. Fairbank showed, using linearization of the Rayleigh-Plesset equation, that increasing the static pressure effectively compresses the microbubble which shifts the resonance frequency of microbubbles toward higher values. Linearization of the Rayleigh-Plesset equation is equivalent to re-
stricting the behaviour of a microbubble to that of a simple harmonic oscillator. It yields important analytic information otherwise unavailable through the complete Rayleigh-Plesset model, although the model fails at explaining higher order phenomena such as harmonic generation. Nevertheless, linearization predicts a small shift in the resonance frequency of unshelled microbubbles which was observed by Fairbank for 30-40 $\mu$m diameter microbubbles and hydrostatic pressures changes of about 0.2 atm. Ishihara et al. [20] validated this later, using a similar experiment. Although Shankar et al. [21, 22] demonstrated that the sensitivity in detecting the resonance frequency in bubble populations can be increased using a dual frequency acquisition system, interpolating Fairbank’s and Ishihara’s results, the resonance frequency shifts for 10 mmHg would a priori seems too small for clinical application.

Physiological conditions constrains microbubbles to have diameters smaller than 10 $\mu$m, a size scale under which unshelled gas microbubbles dissolve in blood within few milliseconds, making unshelled agents unusable for clinical purpose. For this reason,
microbubbles are coated with a shell. Shelled microbubbles have a much higher half-life than unshelled microbubbles, but behave differently than free gas microbubbles due to their shell properties. Different approximating scheme/models are used depending upon the coating (phospholipid, protein, etc.). One of the first models was proposed by De Jong et al. [23] and accurately described the behaviour of first generation protein shell bubbles by using viscous and surface tension correction terms to the Rayleigh-Plesset equation. Most of the 3rd generation contrast agents commercially available now are made of a very organized monolayer of phospholipid which cannot be accurately represented by the De Jong model. Marmottant et al. [24] introduced a more accurate model based upon the buckling behaviour of phospholipid monolayers that predicts low acoustic-amplitude subharmonic emission (i.e., a component at half of the fundamental frequency). It has been shown by Bouakaz et al. [25] and Tickner et al. [26] that it is possible, using the appropriate shell structure, to correlate the dissolution time or stability of microbubble to blood pressure.

It has been observed recently by Shi, Forsberg et al. [27, 28] and Andersen et al. [29] that subharmonic emissions of phospholipid microbubbles are dependent upon blood pressure. In particular, Frinking et al. [30] showed experimentally that phospholipid microbubbles increase their subharmonic power considerably when the ambient overpressure forces the bubble to enter a buckled state (surface-tension free); a 17 dB increase in subharmonic amplitude after applying 60 mmHg has been reported. It has been suggested based upon these results that subharmonic amplitude can be a good indicator of the local relative blood pressure.

It is also expected, based on linear theory, that buckling should also greatly affect the resonance frequency of phospholipid microbubbles (chapter 2), an idea which has attracted little attention since the work of Fairbank et al. [19] and Ishihara et al. [20]. Although changes in the resonance frequency will most likely be lesser than the drastic 17dB variation in the backscattered echo described by Frinking et al. [30], backscattered
amplitude measurements are strongly dependent upon the microbubble shell properties, the insonification frequency and the the attenuating medium used (tissue), making any gauge blood pressure measurement at best very difficult. Using the resonance frequency of the fundamental to measure gauge blood pressure in the portal vein seems to provide a priori a more tractable and reproducible method.

1.5 Research question and structure of thesis

The objective of this thesis is to determine whether or not it is feasible to measure gauge blood pressures using microbubbles’ fundamental mode of scattering, in combination with the effect of shell buckling, and to determine whether or not a 10 mmHg pressure resolution (±5 mmHg), required for portal vein hyperpressure diagnosis, can be achieved. To understand conceptually the effect of shell surface tension, two different models will be compared: a De Jong (viscoelastic) surface tension and a Marmottant buckling surface tension which apply respectively, to a protein and a phospholipid shell. A summary of uncoated and shelled microbubbles, the linearized properties of microbubbles as well as the full numerical solutions of the Marmottant equation for the parameters of interest will be conducted in chapter 2. These simulations predict interesting properties that will be investigated in an in-vitro setup for Optison and Borden (phospholipid) microbubbles. Chapter 3 will study the statistical effects of bubble population on the efficiency of the method of using microbubbles to measure blood pressure, considering the problem of inhomogeneity of physical properties (size, elasticity, initial surface tension). Chapter 4 will contain a summary of the thesis results, and a comment on the feasibility of using microbubbles to measure blood pressure with an accuracy 10 mmHg.
Chapter 2

The effect of blood pressure on microbubble resonance

2.1 Theory: Physics of microbubbles

2.1.1 Unshelled microbubbles: the Rayleigh-Plesset equation

The question of explaining the behaviour of bubbles under various boundary conditions is an almost 100-year-old problem. It was observed at the beginning of the 20th century that the creation of low pressure regions by the fast rotation motion of pumps and propellers caused the formation of unstable air bubbles, which damaged the propeller itself as the bubble collapsed, a process now known as inertial cavitation. Lord Rayleigh made a first attempt to explain bubble inertial collapse in his 1917 paper [31]. The phenomenon of stable cavitation of a vapour-filled bubble was later explained by Plesset [32]. This initial form of the Rayleigh-Plesset equation neglected the effect of viscosity as well as the compressibility of the surrounding medium, which were later introduced by Herring, Keller and Miksis [33], and Gilmore and Aklichev (see Emmer [34]) . In particular, the model from Keller and Miksis [33] was used as a starting point for the Marmottant equation that will be introduced later. The Keller-Miksis [33] equation of
motion of a bubble is:

\[
\rho_l \left( R \ddot{R} + \frac{3}{2} \dot{R}^2 \right) = \left( p_o + \frac{2\sigma}{R} \right) \left( \frac{R_o}{R} \right)^{3\kappa} \left( 1 - \frac{3\kappa}{c} \dot{R} \right) - \frac{2\sigma}{R} - \frac{4\mu_l \dot{R}}{R} - P_o - P(t) \quad (2.1)
\]

where \( R(t) \) is the instantaneous microbubble radius, \( R_o \) is the equilibrium radius, \( \kappa \) is the polytropic constant, \( \rho_l \) is the liquid density, \( \mu_l \) is the liquid viscosity, \( P_o \) is the static fluid pressure, \( \sigma(R) \) is the bubble-liquid interface surface tension and \( P(t) \) is any other applied pressure field (e.g., ultrasound).

**Derivation of the incompressible, non-viscous Rayleigh-Plesset equation**

The derivation follows closely the approach developed in Leighton [35] and Emmer [34]. It will be assumed in this derivation that the bubble oscillates spherically, i.e. the wavelength of the incident sound plane wave is much greater than the bubble size (Rayleigh condition), so that non-spherical modes can be neglected. The energy of the liquid moving due to the oscillation of a bubble is:

\[
K_{\text{liquid}} = \int_{\text{liquid}} \frac{1}{2} \rho v^2(\vec{r}) dV = \frac{1}{2} \rho \int_R^\infty \dot{r}^2 4\pi r^2 dr \quad (2.2)
\]

where \( R \) is the bubble radius, \( \rho \) is the density of the fluid and \( v(\vec{r}) \) is the velocity of the fluid at location \( \vec{r} \). Assuming in addition that the liquid is incompressible, the flux through the hollow sphere formed by the radius \( r \) and \( R \) (\( r > R \)) is constant (\( 4\pi r^2 \dot{r} = 4\pi R^2 \dot{R} \)) which gives \( \frac{\dot{r}}{R} = \frac{R^2}{r^2} \). Without viscous loss, the kinetic energy is equal to the change of work from the pressure at infinity and the pressure at the bubble wall, such that:

\[
\Delta W = K_{\text{liquid}} \quad (2.3)
\]

\[
\int_{R_o}^R (p_l - p_\infty) 4\pi R^2 dR = 2\pi \dot{R}^2 R^3 \quad (2.4)
\]
Expressing the previous equation in differential form instead of integral notation yields:

\[
\frac{p_t - p_\infty}{\rho} = \frac{3}{2} \ddot{R}^2 + R \dddot{R} \tag{2.5}
\]

The pressure inside of the liquid at the boundary of the bubble (left hand term in equation 2.5) is found by using Newton’s 2nd law and thermodynamics. The total internal pressure inside of the bubble \(p_i\) will be the sum of both the gas pressure \(p_g\) and the vapour pressure \(p_v\) (which is usually small and can often be omitted). Also, \(p_i\) must be equal to the pressure just outside of the bubble \(p_l\) plus the surface tension of the interface \(p_\sigma = \frac{2\sigma}{R}\):

\[
p_g + p_v = p_l + \frac{2\sigma}{R} \tag{2.6}
\]

When the bubble is in equilibrium (no acoustic pressure), \(p_t \to p_o\) and:

\[
p_{g,e} = p_o + \frac{2\sigma}{R_o} - p_v \tag{2.7}
\]

The gas expansion can be modelled by the polytropic gas law \(p_g \propto R^{-3\kappa}\) where \(\kappa\) varies depending upon whether isothermal or isentropic conditions are used (frequency dependent). Combined with the previous result, the pressure at the bubble wall is determined to be:

\[
p_t = \left( p_o - p_v + \frac{2\sigma}{R} \right) \left( \frac{R_o}{R} \right)^{3\kappa} + p_v - \frac{2\sigma}{R} \tag{2.8}
\]

Since \(p_\infty = p_o + P(t)\), where \(P(t)\) is the driving acoustic field, we obtain the Rayleigh-Plesset equation

\[
\rho_l \left( R \dddot{R} + \frac{3}{2} \dot{R}^2 \right) = \left( p_o + \frac{2\sigma}{R} \right) \left( \frac{R_o}{R} \right)^{3\kappa} + p_v - \frac{2\sigma}{R} - p_o - P(t) \tag{2.9}
\]

### 2.1.2 Shelled microbubbles: viscoelastic and phospholipid shells

The gas pressure inside of the bubble is typically greater than the partial pressure of the dissolved gas in the liquid phase due to surface tension. As a result, a bubble will
constantly shrink in size because of diffusion from the gas core. Using the equation from Epstein and Plesset [36] it can be shown that the typical lifetimes of 2.5 µm and 5 µm microbubbles are 95 ms and 6.7 s [37], respectively, at room temperature and ambient pressure, making such bubble of very limited clinical use. For this reason, clinical microbubble contrast agents are surrounded by a shell (e.g., protein, phospholipid) which considerably limits the gas leakage rate and by consequence improves the stability of microbubbles in liquid.

Figure 2.1: A schematic view of a shelled microbubble. Shells, through different mechanisms depending upon the shell nature, considerably increase the lifetime of microbubbles.

The physics of shelled microbubbles (figure 2.1) are much more complex. Whereas the Rayleigh-Plesset equation explains well the cavitation of gas bubbles, many models compete to describe the behaviour of shelled microbubbles. De Jong (1994) was a pioneer in ultrasound contrast agent modelling and proposed the addition of two ad hoc terms to the Rayleigh-Plesset equation to compensate for the shell friction and elasticity respectively. Since then, many other individuals developed other shell models, notably Church et al. [38], Hoff et al. [39], Morgan et al. [40], Khismatullin et al. [41], Chatterjee et al. [42], Allen et al. [43], Sarkar et al. [44], Marmottant et al. [24], Doinikov et al. [45], Stride et al. [46] and Tsiglisfis et al. [47]. Although each model is different, by the shell properties studied and the approach used, the basic idea suggested by De Jong is often followed.
In this thesis, we will use both the viscoelastic model from De Jong and the buckling dominated model from Marmottant, which both rely on a Keller-Miksis type Rayleigh-Plesset equation (i.e., compressibility included) and on additional surface tension and shell damping contributions. It can be mathematically shown that both models follow the same equation of motion, but with different expressions of $\sigma(R)$:

$$\rho_l \left( \ddot{R} \dddot{R} + \frac{3}{2} \dot{R}^2 \right) = \left( p_o + \frac{2\sigma(R_o)}{R} \right) \left( \frac{R_o}{R} \right)^{3\kappa} \left( 1 - \frac{3\kappa}{c} \ddot{R} \right) - \frac{2\sigma(R)}{R} - 4\mu_l \dot{R} - 4\kappa_s \dot{R} R \left( \frac{R_o}{R} \right) - P_o - P(t)$$ (2.10)

The De Jong viscoelastic model assumes a constant shell elasticity $\chi$, imposing the following form for the surface tension:

$$\sigma(R) = \sigma(R_o) + 2\chi \left( \frac{R}{R_o} - 1 \right)$$ (2.11)

It expresses well the behaviour of simpler microbubbles, like protein microbubbles, but fails at explaining thoroughly the response of phospholipid shell microbubbles. Typical phospholipid microbubbles increase the lifetime of microbubbles by reducing the surface tension $\sigma$ between the gas core and the liquid, which limits gas diffusion. In Marmottant’s model, the previously ad-hoc elasticity term is now included in a more physically meaningful bubble radius-dependent surface tension $\sigma(R)$ interaction (and therefore a radial-dependent elasticity $\chi(R)$), since the surface tension of a biphospholipid interface is strongly dependent upon the effective area of the molecule [48]. The Marmottant equation of motion is written as:

$$\sigma(r) = \begin{cases} 0 & \text{if } r < r_{\text{buck}} \\ \chi_o \left( \frac{r^2}{r_{\text{buck}}^2} - 1 \right) & \text{if } r_{\text{buck}} < r < r_{\text{ruptured}} \\ \sigma_w & \text{if } r > r_{\text{ruptured}} \end{cases}$$ (2.12)
Chapter 2. The effect of blood pressure on microbubble resonance

This form of \(\sigma(R)\) is estimated from the observation that a phospholipid bubble buckles if the molecule density reaches a threshold value (corresponding to a high compression state) while it becomes ruptured if stretched past a fixed break-up radius (an example of surface tension and shell elasticity is shown in figure 2.2). This model for \(\sigma\) has 3 degrees of freedom (DOF): \(\chi\), \(r_{\text{ruptured}}\) and \(r_{\text{buckling}}\). For low amplitude oscillations, the bubble lays in an elastic regime where the elasticity (defined as \(\chi = A \frac{d\sigma}{dA}\)) is close to a constant. For low amplitude oscillation, it can be shown that the first order Taylor expansion of \(\sigma(R)\) reduces to the De Jong model.

![Figure 2.2: The model of Marmottant. The surface tension (left) and elasticity (right) of a phospholipid microbubble has 3 regimes: buckling, elastic and free. In Marmottant’s initial model, a piecewise function was used to separate these regimes. \(R_o = 3.0\mu m\), \(R_{\text{buckling}} = 2.92\mu m\), \(\chi_o = 0.55N/m\). (Microbubble images from Sijl [3])](image)

A problem with the Marmottant formulation is that \(\sigma(r)\) is discontinuous both at \(r_{\text{ruptured}}\) and \(r_{\text{buckling}}\), meaning that \(\chi\) does not exist at those points, which is unphysical. To correct this issue, a modified version of \(\sigma(r)\) proposed by Sijl [3] uses quadratic transitional regions \(Y_1, Y_2\) at buckling and rupture. The model is well posed if continuity and differentiability boundary conditions are imposed, leaving again 3 DOF. The Sijl equation of state is shown below:
\[
\sigma(r) = \begin{cases} 
0 & \text{if } r < r_{\text{buck}} \\
\frac{1}{2} \zeta_0 \left( \frac{r}{r_{\text{buck}}} - 1 \right)^2 & \text{if } r_{\text{buck}} < r < r_{\text{Ela}1} \\
2 \chi_0 \left( \frac{r}{r_0} - \delta r \right) & \text{if } r_{\text{Ela}1} < r < r_{\text{Ela}2} \\
\sigma_w - \frac{1}{2} \zeta_0 \left( \frac{r}{r_{\text{buck}}} - \frac{r_{\text{ruptured}}}{r_{\text{buck}}} \right)^2 & \text{if } r_{\text{Ela}2} < r < r_{\text{ruptured}} \\
\sigma_w & \text{if } r > r_{\text{ruptured}} 
\end{cases} 
\] (2.13)

Figure 2.3: Sijl buckling model. Quadratic regions $Y_1$ and $Y_2$ are used as transitional regions instead of the sharp edge transitions suggested by Marmottant. (Figure adapted from Sijl [3])

Although $\chi(R)$ and its derivative $\zeta(R)$ are dependent upon the instantaneous radius of the microbubble, $\chi_0$ and $\zeta_0$ are constant, depending only upon the morphology of the microbubble shell. An interesting application of the Sijl model is that, once the 3 DOF of the shell are known, it is possible to predict the exact radius width of the transition zones $Y1$ and $Y2$ directly.
2.1.3 Modelling quasi-static compression of microbubbles

The quasi-static compression of a bubble due to blood pressure, occurring very slowly in time compared to ultrasound frequencies, can be modelled using purely thermodynamic concepts derived in the previous sections, namely polytropic gas law, and pressure equilibrium. Equilibrating the pressure forces inside of the bubble at different compression states yields:

\[
P_{g,e}(P_{o1}) \frac{P_{g,e}(P_{o2})}{P_{o2}} = \left( \frac{P_{o1} + \frac{2\sigma(R_{01})}{R_{01}} - P_v}{P_{o2} + \frac{2\sigma(R_{02})}{R_{02}} - P_v} \right)^{-3\kappa}
\]

(2.14)

where the indices 1 and 2 refer to different states of compression of the microbubble. This equation does not have an analytic solution, but can be easily solved numerically (solution is shown in figure 2.4). The microbubble shell coating usually reduces the effect of static pressure compressions by making the bubble more stiff, but as can be seen in figure 2.4, its effect is negligible compared to the gas thermodynamics for the literature values of \( \chi \) [49].

Figure 2.4: Compression of a 3 \( \mu m \) radius microbubble due to static pressure. The Sijl model was solved both for a viscoelastic shell (solid blue) and a free gas microbubble (dotted green).
2.1.4 Linear approximation of the hydrostatic dependence of microbubble echoes

The equation of motion of microbubbles is a non-linear non-homogeneous ordinary differential equation with no known analytical solution. Although simulations must be used if one wants to understand the exact solution of such an ODE, linearization yields the first order behaviour of microbubbles. In such a scheme, an \textit{ansatz} solution to the Marmottant equation is assumed to be \( R(t) = R_o (1 + x(t)) \) (lowest Taylor expansion) where \( x(t) \ll 1 \) (dimensionless) and \( x(t)^2 \) or higher order terms are then considered negligible.

It is well known \cite{49} (and demonstrated in Appendix A) that a driven damped harmonic oscillator solution is recovered:

\[
\ddot{x} + \omega_o \delta \dot{x} + \omega_o^2 x = \frac{P(t)}{\rho R_o^2} \leftrightarrow X(\omega) = \frac{1}{\rho R_o^2} \frac{P(\omega)}{(\omega_o^2 - \omega^2)^2 + (\delta \omega_o)^2}
\]

\[
\omega_o = \sqrt{\frac{1}{\rho R_o^2} \left( 3\kappa P_o + \frac{2(3\kappa - 1) \sigma_w}{R_o} + \frac{4\chi(R_o)}{R_o} \right)}
\]

\[
\delta = \delta_{\text{radiation}} + \delta_{\text{viscous}} + \delta_{\text{shell}} = \frac{\omega_o R_o}{c} + \frac{4\mu_l}{R_o^2 \rho \omega_o} + \frac{4\kappa_s}{R_o^3 \rho \omega_o}
\]

where \( X(\omega) \) is the Fourier transform of \( x(t) \), \( \omega_o \) is the natural resonance frequency and \( \delta \) is the total damping in the system. The natural resonance frequency \( \omega_o \) is not the resonance frequency of a damped system defined as the maximal response \( \left( \frac{dX(\omega)}{d\omega} = 0 \right) \).

Since the linearized backscattered pressure is \( P_{\text{scat}}(t) \cong \frac{1}{\rho R_o^2} \dot{R}(t) \), the radial response resonance \( \omega_{\text{res},R} \) and scattered pressure resonance \( \omega_{\text{res},P} \) will also have slightly different frequencies and it can be shown that the associated resonance frequencies for the radius \( \omega_{\text{res},R} \) and the pressure response \( \omega_{\text{res},P} \) are:

\[
\omega_{\text{res},R} = \sqrt{1 - \frac{\delta^2}{2}} \omega_o
\]

\[
\omega_{\text{res},P} = \frac{1}{\sqrt{1 - \frac{\delta^2}{2}}} \omega_o
\]

The hydrostatic pressure has both explicit (in \( P_o \)) and implicit contributions (due to the compression of the equilibrium radius \( R_o \)) on the resonance frequency. In addition,
phospholipid coating elasticity depends upon the bubble radius, an effect which needs to be accounted for.

Viscolelastic model

As mentioned previously, viscoelastic microbubble shells have a constant elasticity with respect to the equilibrium radius $R_o$ (and therefore $P_o$) and viscosity, making their response to blood pressure more tractable. The linearized response of viscoelastic microbubbles versus frequency and hydrostatic pressure was calculated using equation 2.15 (figure 2.5).

Figure 2.5: Normalized linearized backscattered pressure response from a 3.0 µm radius microbubble versus frequency and gauge hydrostatic pressure computed from equation 2.15. $P_{ac} = 10kPa$, $\chi = 0.54N/m$

Hydrostatic pressure shifts the resonance peak toward higher frequencies, an effect predicted by Fairbank [19] for the case of free gas bubbles. The coating merely increases the net elasticity $\chi$, therefore adding a constant shift to the resonance frequency while the shell viscosity increases the overall damping of the system. As for unshelled bubbles, gas dynamics will be responsible for the static pressure-dependent behaviour of viscoelastic
Figure 2.6: Two methods used to measure blood pressure based on linear theory. Left: relative measurement based on backscattered amplitude (blue: 1.24 MHz, red: 2.49 MHz, green: 6.00 MHz). Right: Gauge pressure measurement using resonance frequency $P_{acc} = 10kPa$

These results suggest that two different techniques could be used to measure blood pressure:

- **Relative manometry**: The backscattered pressure amplitude becomes strongly dependent upon hydrostatic pressure when looking at a fixed frequency close to the resonant peak. Considering a linear model and a 3 $\mu m$ radius microbubbles, relative changes as high as 70% are observed for 750 mmHg changes in static pressure for frequencies close to resonance, while the amplitude changes by only 9% at frequencies much higher than resonance. Gauge pressure values cannot be obtained using this technique.

- **Gauge manometry**: Accurate measurement of the resonance frequency can be converted to an absolute pressure value, i.e. with respect to pure vacuum, by using linear theory as an inversion curve $(f(P_o) \rightarrow P_o(f))$. This measurement can be translated to gauge pressure as long as the atmospheric pressure is known. This is the principal method focused on in this thesis.
A limitation is that the increase of the resonance frequency of viscoelastic microbubbles varies very little over the biological pressure range of interest (≈ 0.5% over 10 mmHg), and may be difficult, if not impossible, to observe considering all other external sources of noise occurring in a realistic clinical setup.

**Phospholipid model**

The elasticity $\chi = A \frac{d\sigma}{dA}$ of a lipid coated bubble is a strong function of the bubble radius. An increase of the static pressure compresses a bubble, decreasing its equilibrium radius. As long as the bubble’s equilibrium radius stays far from the buckling radius, it will behave similarly to a viscoelastic bubble (changes in $\chi$ will be small) and therefore the resonance frequency will slowly increase due to gas dynamics. Yet when $P_o$ is high enough such that the buckling radius is reached, the bubble will undergo buckling, reducing the effective elasticity of its shell to zero and considerably reducing the resonance frequency. Those regions of high fluctuations happen within the transitional regions $Y_1$ from the Sijl model ($Y_2$ may become important if static pressure is decreased), within which the bubble should be a much more sensitive manometer than protein-shell microbubbles. Assuming the bubble properties shown in figures 2.2, it can be shown that the 0-120 mmHg region is the viscoelastic regime, the 120-173 mmHg region is the buckling transition, and the 173-750 mmHg region is the buckled-state regime (refer to figure 2.7). The microbubble resonance frequency undergoes at steep change in the transitional region varying between 1.9 MHz to 1.22 MHz in a mere 53 mmHg pressure change, and the buckling bubble is roughly 40-120 times more sensitive to hydrostatic pressure than the viscoelastic microbubble within these limits.

### 2.1.5 Summary and limits of linear models

Linear theory shows that phospholipid microbubbles respond differently to static pressure than other bubbles (protein and unshelled) due to their buckling dynamic. Namely,
Figure 2.7: Elasticity (left) and resonance frequency (right) versus hydrostatic pressure for a buckling bubble using parameters from figure 2.2 (taking the first derivative of the surface tension). Drastic changes in the elasticity occur when static pressure forces the microbubble to enter into a transitional state. This causes a steep drop of the resonance frequency estimated using equation 2.15.

Phospholipid microbubbles steeply decrease their resonance frequency, while protein shell microbubbles slowly increase their resonance frequency with increasing blood pressure. Yet, linear solutions have important limitations. It is known experimentally that microbubbles produce harmonics and subharmonics while linear scatterers emit only at the fundamental frequency (1st harmonic). Moreover, linear models do not account for any finite size oscillation effects associated with the nonlinear Rayleigh-Plesset ordinary differential equation.

2.2 Methods

Linearization, although very simple, predicts that a protein shell microbubble should respond very weakly to blood pressure, while phospholipid microbubbles should lower their resonance frequency considerably with increasing blood pressure. Due to the inherent non-linear properties of microbubbles, it is still unclear if this effect, as predicted by
theory, will be present in simulations and in an experimental pulse-echo setup.

2.2.1 Simulation

The pulse sequence used is a series of long narrowband pulses spanning a frequency range instead of a single broadband pulse that is typically used to measure the transfer function of transducers. Such a method is time and resource intensive, but reduces the risk of frequency coupling artifacts inherent to the non-linear dynamics of microbubbles. The acoustic pressure, or driving pressure $P_{ac}$, is also left as a variable ranging from 1 kPa to 350 kPa to study the nonlinear hydrostatic pressure-dependent behaviour of microbubbles. The Marmottant model (equation 2.10) is then solved for the radial response $R(t)$ using the MATLAB ODE solver for both shell models (microbubbles parameters from Van der Meer et al. [49]). Finally the scattered pressure emitted, $P_{scatt}$, is calculated using conservation of mass/momentum assumptions (for a derivation refer to [35]):

$$P_{scatt}(r, t - \frac{r}{c}) = \frac{p}{r} \left( R(t)^2 \ddot{R}(t) + 2R(t)\dot{R}(t)^2 \right)$$

(2.17)

The previous equation describes the active scattering contribution from microbubbles due to the coupling of the bubble motion with the surrounding liquid. It was demonstrated by Leighton [35] that the passive contribution related to the impedance mismatch can be safely neglected. The Bernouilli pressure, or kinetic wave, was also neglected since it decays as $\frac{1}{r^4}$. $P_{scatt}$ is Fourier transformed, producing a spectrum of sharp peaks at the harmonic and subharmonics frequencies (due to narrowband excitation) and the amplitude of each peak is extracted using bandpass filters. We emphasize again that each time the Marmottant ODE is solved, the centre frequency of the pulse $\omega$, the acoustic pressure $P_{ac}$ and the static pressure $P_o$ are kept fixed. For a systematic narrowband study of $P_{scatt}(\omega, P_{ac}, P_o)$, the ODE needs to be solved $n^3$ times where $n$ is the desired point density. A schematic representation of the process is shown in figure 2.8
Figure 2.8: A schematic of the simulation process used. The boxes in orange correspond to the studied variables ($P_{ac}$, $\omega$ and $P_o$). The process must be repeated to cover the full parameter space.

### 2.2.2 Experiment

The experiment was performed using a single spherically focused transducer centred at 3.5 MHz with 85% bandwidth. A pulse-echo setup was used (A-mode) where the microbubbles were imaged through an acoustically transparent layer. The acoustic chamber was connected to a manometer and an adjustable pressure reservoir (a 60ml needle filled with fluid connected to the system which was slowly compressed or expanded to adjust the pressure value). The frequency response was measured over the 0-150 mmHg static pressure range (depending upon the stability of the microbubbles). The static fluid pressure was kept fixed during each spectrum measurement. Similarly to the simulation pulse sequence a series of narrowband pulses, consisting of 50 17-cycles pulses covering the 1MHz to 6MHz range, were produced instead of a typical short broadband pulse. The experiment was conducted on 3 different microbubble samples:

- **Borden type phospholipid microbubbles**: A surfactant solution of 1,2-distearoyl-sn-Glycero-3-phosphocholine and polyoxyethylene (40) stearate was prepared according to the recipe from Borden. The solution was then sonicated at high frequency while in a saturated perfluorobutane atmosphere, creating microbubbles of variable diameters between 1 to 10 $\mu m$ filled with perfluorobutane.

- **Centrifuged Borden microbubbles**: As sonicated microbubbles typically have
different radii and shell properties, a centrifuge can be used to reduce the spread of the microbubble size distribution. More details about the technique will be given in the next chapter.

- **Optison microbubbles**: A clinical protein-shell microbubble contrast agent.

![Experimental setup used for the measurement of the hydrostatic pressure-dependent response of microbubble populations.](image)

Figure 2.9: Experimental setup used for the measurement of the hydrostatic pressure-dependent response of microbubble populations. A 3.5 MHz 85% bandwidth spherically focused transducer was used at low acoustic amplitude.

Enough measurements were compounded to minimize the effect of statistical speckle (a slow rotation velocity magnetic stirrer ensured that each measurement was independent). The signal was time gated at the focus of the transducer. In addition to the speckle effect, back-scatter spectroscopy experiments were limited by the bandwidth of the transducer. Each transducer has a particular frequency response curve, or transfer function \( H \), which will affect both the transmitted \( H_T = \frac{P_{\text{transmitted}}}{V_{\text{input}}} \) and received \( H_R = \frac{V_{\text{received}}}{P_{\text{input}}} \) signals. Under the reciprocity approximation, it can be shown that \( H_T \propto H_R \), where the proportionality constant is known as the reciprocity factor [50]. Sijl \textit{et al.} [3] measured the deviation due to reciprocity approximation for a single element transducer. Maximal deviations of 15% compared to the exact method were observed by the authors. In particular, deviations become non-negligible if the transducer is non-linear, i.e. if a high
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Figure 2.10: A) Calibration using a quartz plate. Linear theory yields \( V_{\text{out}}(t) = V_{\text{in}}(t) \ast H_T \ast H_R \). B) A typical measurement on a microbubble population. This is exact only if we consider linear propagation (including linear scattering), and approximate when considering non-linear scatterers: \( V_{\text{out}}(t) \approx V_{\text{in}}(t) \ast H_T \ast H_B \ast H_R \)

voltage is applied. We avoided such cases in our experiment and kept the applied voltage to the transducer low.

The frequency dependent response is very strong, even for high bandwidth transducers, and the microbubble scattered spectrum needs to be normalized by the transducer transfer function. Calibration was accomplished by measuring the backscattered reflection from a quartz plate. To minimize the error sources, the calibration was done with the same narrowband pulse sequence as the one used for microbubble insonification at the focus of the transducer. Only frequencies within the FWHM of the transducer transfer function were considered.

It is straightforward, under linear signal propagation conditions, to compute the transfer function of the bubble which turns out to be \( H_B(\omega) = \frac{V(\omega)}{V_{\text{calib}}(\omega)} \) (schematic shown in
Figure 2.10). If the nonlinearity of the bubble becomes strong, complexities arise and an exact transfer function does not exist. Though, under the assumption that the transducer behaves linearly and under reciprocity, and the microbubble weakly non-linearly, we will define a transfer function, based on linear theory, such that:

\[
H_B(\omega, \omega_s) = \frac{P_{\text{scat}}(\omega_s)}{P_{\text{input}}(\omega)} = \frac{V_{\text{out}}(\omega_s)H(\omega)}{V_{\text{calib}}(\omega)H(\omega_s)}
\]  

(2.18)

where H refers to either \(H_R\) or \(H_T\) (which are equals since reciprocity is assumed) and a second frequency \(\omega_s\) (scattering frequency) has been introduced since we have relaxed the condition of linear scattering from microbubbles, such that the principle can be applied to any harmonic. In particular, the following expressions are obtained for fundamental and second harmonic:

\[
H_B(\omega, \omega) = \frac{V_{\text{out}}(\omega)}{V_{\text{calib}}(\omega)}
\]

\[
H_B(\omega, 2\omega) = \frac{V_{\text{out}}(2\omega)H(\omega)}{V_{\text{calib}}(\omega)H(2\omega)} = \frac{V_{\text{out}}(2\omega)}{\sqrt{V_{\text{calib}}(\omega)V_{\text{calib}}(2\omega)}}
\]  

(2.19)

The calibration voltage response \(V_{\text{calib}}(\omega)\) is shown in figure 2.11. The response was computed for different values of applied voltage to monitor the possible deviations attributed to non-linear response from the transducer. Over the range from 11V to 70V, the transfer function was reproducible to 4% and we can safely argue that the transducer was linear. The bandwidth of the transducer was also evaluated using these measurements.

The transfer function \(H_B\) is also affected by the frequency-dependent absorption of the medium \((\alpha = \alpha_0 f^n, \text{ where } \alpha \text{ is the attenuation coefficient in } \text{db/cm and } \alpha_0 \text{ is a constant associated with the medium})\). This can distort the \(H_B\) spectrum and possibly shift the resonance frequency. This effect is negligible for water (used in this experiment), where \(\alpha\) varies from 0.002 dB/cm to 0.03 dB/cm [51] from 1 MHz to 5 MHz respectively, but become significant for tissues where \(\alpha\) is typically higher than 1 dB/cm.
Chapter 2. The effect of blood pressure on microbubble resonance

2.3 Results and discussion

2.3.1 Simulations of the hydrostatic pressure dependence

In the theory section, we presented the Rayleigh-Plesset theory of a shelled microbubble and demonstrated how linearization infers information about their hydrostatic dependent behaviour. Here we will simulate directly the response of individual microbubbles by solving numerically the Rayleigh-Plesset equation for different surface tension models.

Viscoelastic microbubbles

A viscoelastic bubble denotes a bubble exhibiting both an elastic (constant elastic modulus) and a viscous behaviour. Protein shell microbubbles follow this approximation. A typical simulation of a viscoelastic bubble, according to the De Jong model, is shown in figure 2.12 for the bubble radial response and in figure 2.13 for the pressure response. A first observation is that indeed the linear model is recovered for low acoustic pressures.
When the acoustic pressure reaches about 125 kPa, the backscattered pressure does not increase linearly with $P_{ac}$ anymore. As another consequence of nonlinearity, the resonance frequency is artificially upshifted from 1.8 MHz to 2.0 MHz (a 10 % change) over the 350 kPa range. The linear model holds unexpectedly well for acoustic pressures up to 125 kPa, considering that the second harmonic to fundamental signal from figure 2.13 can reach twice the value of the fundamental signal. This suggests that the relative amount of second harmonic to the total backscattered signal should not be used as an experimental criterion to judge the validity of the linear theory. A better standard would be to look at the point where $O(ax) \neq aO(x)$ for the backscattered amplitude, i.e. the formal mathematical definition of a non-linear system.

Figure 2.12: An example of viscoelastic simulation of the radial response using the full equation of motion for a 3 µm radius microbubble of constant elastic modulus ($\chi = 0.54 N/m$). The vertical axis corresponds to the harmonic fractional change in the bubble radius. Atmospheric pressure (static fluid pressure of 100 kPa, or 0 kPa overpressure) is assumed. Each graph was normalized by $P_{ac}$ such that departure from the linear regime can be clearly observed.
Figure 2.13: Scattered pressure signal (normalized) of figure 2.12 radius signal. Notice that the ratio of the second harmonic to fundamental can be as high as 2, while causing very little deviation of the fundamental from the linear model.

The studied model also suggests that most of the second harmonic signal is emitted when the insonification frequency lies between $\frac{1}{2}f_{\text{res}}$ and $f_{\text{res}}$ (with a maximum at $f_{\text{res}}$), while the maximum second harmonic to fundamental ratio arises exactly at $\frac{1}{2}f_{\text{res}}$.

To investigate the hydrostatic dependent behaviour of viscoelastic microbubbles, figure 2.13 was repeated for increasing values of static pressure. Results are displayed in figure 2.14 and figure 2.15. The same effects as those predicted by the linear theory are observed: strong hydrostatic pressure-dependent amplitude modulations close to resonance, and the resonance frequency is slowly shifted toward higher values as static pressure is increased. In the non-linear scattering regime of the bubble, small deviations from the linear model prediction of the resonance frequency are observed (4% to 10% discrepancies). The resonance frequency is then increased roughly by constant offset, which may be attributed to an artificial increase of the shell elasticity. These finite size effects are nevertheless minor and can be compensated for post-calibration.
Figure 2.14: Simulation of the variation of the echo amplitude (normalized) with respect to static pressure of a viscoelastic bubble for both fundamental (left) and second harmonic (right) using the full equation of motion (3 $\mu$m radius, $\chi = 0.54 N/m$)
Figure 2.15: Simulation result for the backscattered amplitude of a single-protein shell microbubble (left) (blue: 1.8 MHz , green: 2.8 MHz , red: 6.00MHz ) and the variation of the resonance frequency with blood pressure using the full non-linear equation of motion. At low acoustic pressure ($P_{ac} = 3\text{kPa}$, blue), the resonance frequency changes by 0.008 MHz per 10 mmHg while it changes by 0.006 MHz per 10 mmHg at $P_{ac} = 350\text{kPa}$ (green)

**Phospholipid shell microbubbles**

Phospholipid bubbles cannot be considered purely as viscoelastic at large driving pressures because they buckle and rupture at precise radii thresholds, and the full Marmottant surface tension model must be used. Simulations of the Marmottant equation of motion with respect to frequency, acoustic pressure and static fluid pressure are shown in figure 2.16 and 2.17. At low acoustic pressures, simulation agree well with linear theory; the resonance frequency decreases fast as the transition regime is reached. Striking discrepancies arise when the resonance frequency is considerably downshifted, as the finite oscillations of bubbles reach the buckling radius (figure 2.16). Such behaviour is not predicted by linear theory, but a qualitative explanation is that as a bubble stays a fraction of its oscillation in buckled state, it will experience a lower net elasticity $\chi$. This happens at a fixed $P_{ac}$ threshold over which the microbubble becomes a very poor manometer (refer to figure 2.17). In the studied case, since the elastic regime covers a very narrow radius span, a shift is observed for acoustic pressures as low as 40 kPa. This
seems to agree with the results from Overvelde et al. [52]. Figure 2.7 will only be valid for low acoustic pressure (< 40kPa in this case) where finite oscillation effects can be safely neglected. This transition occurs at progressively lower acoustic pressures as the microbubble’s initial radius gets closer to the buckling radius. Since $\sigma \in [0, \sigma_w]$, the lower the shell elasticity $\chi$, the bigger the viscoelastic radius span.

Similarly to the viscoelastic microbubbles, a second harmonic peak arises at the fundamental resonance frequency for moderate to high acoustic pressures (figure 2.16). This happens closer to the resonance frequency of unshelled microbubbles, due to buckling. A noticeable exception happens when the bubble rest radius is in the transitional regime, where an anomalous peak exists at low acoustic pressure. Since second harmonics are typically not emitted at such low acoustic pressure, this emission peak can be used as a selective marker for transitional buckling. Most of the static pressure effects on phospholipid microbubbles are attributed to the shell dynamics (the radius dependent elasticity in particular), as opposed to the gas dynamics of viscoelastic microbubbles. Due to these shell effects, phospholipid microbubbles are much more sensitive to blood pressure than protein microbubbles.

2.3.2 Relation to subharmonic emission

It has been shown experimentally [30] that the subharmonic response of microbubbles is strongly correlated to blood pressure. Here we will show that the method using the fundamental resonance tracking is associated to subharmonic emission because of buckling. Subharmonics, as opposed to harmonics, are not always a stable mode of oscillation. While harmonics are always present in the microbubble signal (although very weakly at low acoustic amplitude) and will progressively increase in amplitude as driving force is increased, subharmonics are unstable and simply do not exist until a fixed acoustic pressure threshold is exceeded. This threshold is usually high for unshelled and viscoelastic microbubbles, and anomalously low for phospholipid shell microbubbles. To explain this,
Figure 2.16: Simulation of the hydrostatic pressure dependent response from a buckling bubble. The hydrostatic pressures used are respectively -38 mmHg (non-buckled), 0 mmHg (transition), 375 mmHg (buckled) and 750 mmHg (buckled). $R_{\text{bucking}} = R_o = 2.92 \mu m$, $\chi_o = 2.55 N/m$. 
Figure 2.17: Simulation of the effect of pulse pressure on the response of phospholipid microbubbles to static fluid pressure. At low driving pressures, the microbubble responds well to pressure (0.31 MHz per 10 mmHg at $P_{ac} = 3kPa$ and 0.30 MHz per 10 mmHg at $P_{ac} = 30kPa$). Finite oscillations effects cause the microbubble to become a poor manometer over a precise driving pressure (0.02 MHz per 10 mmHg at 40 kPa in this case), at which the microbubble starts to buckle within each oscillation. $R_{buckling} = R_o = 2.92\mu m$, $\chi_o = 2.55N/m$.

Sijl has demonstrated analytically in his work [3] using a second order expansion ($\frac{1}{2}f$ and $f$ harmonic terms around $f = 2f_o$) that the threshold for subharmonic emission is:

$$P_{threshold}^{ac} = P_o\bar{\omega}b \frac{1}{\frac{1}{2} - \frac{\alpha_1 - \frac{1}{2}\tilde{\omega}^2}{\omega_o^2 - \omega^2}} \quad (2.20)$$

where $\alpha_1$ is associated to the shell surface tension first derivative at equilibrium ($\chi(R_o)$) and second derivative at equilibrium $\xi(R_o) = R_o^2 \frac{\partial^2 \sigma(R)}{\partial R^2} |_{R=R_o}$ as:

$$\alpha_1 = \frac{9}{2}\kappa (\kappa + 1) - \frac{\xi(R_o) - 9\chi(R_o)}{P_oR_o} \quad (2.21)$$

b specifies the overall system damping through:

$$b = \frac{2\mu}{R_o\sqrt{\rho P_o}} + \frac{2\kappa_s}{R_o^2\sqrt{\rho P_o}} + \frac{3\kappa}{2c} \sqrt{\frac{P_o}{\rho}} \quad (2.22)$$
and $\tilde{\omega}$ is the non-dimensional frequency such that $\tilde{\omega} = R_0 \omega \sqrt{\frac{\rho}{P_o}}$.

$\xi(R_o)$ becomes high at transition points, reducing considerably the acoustic pressure required for stable subharmonic mode emission (the threshold acoustic pressure is roughly inversely proportional to the second derivative of the surface tension function for large values of $\xi_o$). This explains why subharmonics can exist at low driving pressure in phospholipid microbubbles but not in protein microbubbles. A subharmonic bandpass filter ($f_{filter} = \frac{1}{2}f_{driving} \pm \epsilon$) was used for the simulation data for all regimes. The resulting plots are shown in figure 2.18. As expected from theory, the subharmonic signal is higher in the transition regimes (about 20 times). Moreover, finite oscillation effects do not seem to increase subharmonic emission; a microbubble whose oscillations reach the buckling point does not emit more subharmonic if its equilibrium value is far from the buckling radius. Similarly, fully buckled microbubbles do not emit significant amounts of subharmonic. Therefore, as static pressure is increased and the bubble enters the transition regime, strong subharmonic emissions will coincide with the fast decrease of the resonance frequency of microbubbles.
Figure 2.18: Simulation of the subharmonic component scattered [in Pa] by a microbubble for various shell regimes. Note that the subharmonic pressure amplitude is roughly 20 times higher in the transition regime than all other cases.
2.3.3 Experiment: static pressure response from populations

First, the microbubble echo spectra were measured for increasing values of acoustic pressure. As the acoustic pressure was progressively increased, the resonance frequency peak is shifted from 4 MHz to 2.3 MHz (refer to figure 2.19). This shift occurred because of the buckling of microbubbles (where the microbubbles will experience part of its excitation in a buckled state), and is in agreement with the observations from Overvelde et al. [52]. This is direct experimental evidence that the acoustic pressure, beyond a certain insonification amplitude threshold, affects the response from phospholipid microbubbles.

\[ \text{Fundamental} \quad \text{2nd Harmonic} \]

Figure 2.19: Variation of the backscattered response from filtered microbubbles as the acoustic pressure is increased. The resonance frequency is progressively shifted toward lower values (indicated by an arrow) due to the buckling of phospholipid bubbles. Voltage applied to transducer: dark blue (11 V), green (16 V), red (42 V) and light blue (70 V)

It is also expected that because of their shell, phospholipid coated microbubbles should be much more sensitive to blood pressure than protein coated bubbles. While protein microbubbles slowly increase their resonance frequencies with static pressure, phospholipid microbubbles will decrease their resonance frequency very abruptly within the transition region. Such an effect should still be apparent, though more diffuse, in a polydisperse
population. Each bubble has a different size, elasticity and surface tension, and therefore will buckle at a different pressure value, yet the net decrease of the resonance frequency of the population should still be apparent. Pulse-echo spectroscopy was performed on phospholipid and protein shell microbubbles (using populations of different size distributions). Such measurements are shown in figure 2.20.

![Figure 2.20: Experimental measurement of the static pressure dependent echoes for centrifuge-filtered Borden microbubbles.](image)

The resonance frequency was extracted from each measurement and plotted versus the hydrostatic pressure (refer to figure 2.21). The error bars in figure 2.21 are associated to the uncertainty in determining the resonance frequency from each response peak as shown in figure 2.20. The magnitude of the error increases when using microbubbles having a poorly resolved resonance peak. The resonance frequency of both size filtered and non size-filtered microbubble populations was considerably reduced as the static pressure was increased, while no significant shift in the resonance frequency of protein microbubbles was detected. Shell effects dominated the static pressure response of microbubbles to the point that significant shifts were observed within the target clinical pressure range (∼ 0.15 to 0.20 MHz within 10 mmHg (refer to figure 2.21 and table 2.1) even for polydisperse
microbubbles. This confirms that phospholipid microbubbles are sensitive enough to permit blood pressure measurement.

![Graph showing the variation of resonance frequency versus static pressure for different microbubble shells.]

Figure 2.21: Experimental pulse-echo measurement of the variation of the resonance frequency versus static pressure for different microbubble shells. Green: Borden centrifuge size filtered. Blue: Borden native. Red: Optison

<table>
<thead>
<tr>
<th>Microbubble type</th>
<th>Maximal resonance frequency shift [MHz] per 10 mmHg</th>
<th>Estimated experimental error [MHz]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optison</td>
<td>0.03</td>
<td>0.1</td>
</tr>
<tr>
<td>Borden narrow size</td>
<td>0.20</td>
<td>0.1</td>
</tr>
<tr>
<td>Borden broad size</td>
<td>0.15</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Table 2.1: Sensitivity of different bubble populations’ resonance frequencies to static pressure variations. Values obtained from data shown in figure 2.21. While the proposed technique is sensitive enough for application in portal vein pressure measurements, it still suffers from some reproducibility issues related to large experimental errors.
In addition to the large estimated experimental error, the centrifuge and native curves in figure 2.21 are not identical, introducing the important question of reproducibility between different population measurements. In particular, at 0 mmHg the centrifuge filtered bubbles had a higher resonance frequency than native microbubbles. Though as the static pressure was increased between 25-100 mmHg, the resonance frequency dropped faster for native phospholipid microbubbles than centrifuged filtered microbubbles. It is expected that these variations were due to the statistical spread of the bubble radius and shell properties (such as surface tension).

2.4 Summary and conclusion

It has been shown in this chapter through theory and simulations of the bubble radial equation of motion that buckling-type phospholipid microbubble resonance frequencies are much more dependent upon blood pressure (40 to 120 times depending upon bubble shell parameters) than protein shell microbubbles, which behave closely to purely viscoelastic microbubbles. This is true as long as the acoustic pressure is kept below a fixed threshold, where the bubble will start to undergo periodic buckling due to finite size oscillations.

Moreover, experiments suggest that the method should be sensitive enough for clinical applications, in particular in portal vein hyper-pressure diagnosis, since variations of the resonance frequency ranging from 0.15MHz to 0.20 MHz per 10 mmHg have been measured for Borden phospholipid microbubbles. The resonance peaks of sonicated microbubbles are poorly resolved (low quality factor), introducing an uncertainty in the precise value of the resonance frequency affecting the reproducibility of the technique. Using different radius-size populations of microbubbles (centrifuge versus native) also causes an even higher systematic error. The next chapter will focus on understanding the role of bubble population statistics in the reproducibility of blood pressure measure-
ments, and determining the required conditions that a bubble population must satisfy in order to minimize these sources of error.
Chapter 3

The statistics of microbubble population response

3.1 Introduction

In chapter 2, the required conditions for a single microbubble to be an efficient local manometer were investigated. In a realistic clinical setup, we rarely deal with the scattering of single microbubbles, but with an ensemble of microbubbles. In addition to a spatial distribution causing speckle, most commercially available ultrasound contrast agents also contain microbubbles of different sizes (probability distribution $P_R$) and shell physical properties (in particular the surface tension at rest with probability distribution $P_\sigma$). It was observed previously in figure 2.21 that increasing the spread in the size distribution of microbubbles in particular (through $P_R$) affects the resonance frequency of microbubbles, which causes an erroneous reading of blood pressure. Similarly, it is expected that increasing the spread in $P_\sigma$ would affect the accuracy of the proposed method. In this chapter, we would like to quantify the limitations imposed by speckle on our technique, as well as the specifications of a microbubble population that would minimize statistical variation.
Although it is possible to simulate the signal from a bubble population by using a Rayleigh-Plesset type model for each bubble, the process is usually time consuming since the ODE must be solved for every bubble and post-processed by using additivity criteria. The approach we will use here is statistical. The idea is not new; it is fairly common in physics to switch from an element base to an ensemble type approach as the size of a population increases. The driving factor is that we want to develop more physical insight than rigid exactness to simplify the analysis. The first step will be to recall a statistical theory, based upon red blood cell scattering, which models our system well for the studied concentrations, assuming continuous plane wave excitations. We will then push this model to incorporate broadband and nonlinear pulses scattering from microbubbles, and investigate its validity. Three properties of this model will then be studied: signal-to-noise ratio, the effect of size population and the effect of variation in shell properties.

3.2 Theoretical formulation of population response

3.2.1 Continuous wave linear approximation model

The principle of superposition is a strong axiom in physics. For many physical quantities, combining two waves can be done through simple addition. Namely, it applies to displacement waves, velocities (in a Galilean scheme), forces and pressure waves. In this section, we will develop an analytical model of population scattering. We will now assume a simplified scheme with:

- linear scattering conditions, applied both to the radius and scattered pressure response of the microbubble;

- a continuous planar wave driving pulse (CW).
Figure 3.1: Schematic view of the bubble population setup, including axis and reference centre.

A schematic view of this thought experiment is shown in figure 3.1. $\vec{R}$ refers to the vector between a bubble and the observation point, $\vec{r}$ is the position vector of the bubble in the reference frame and $\vec{r}_o$ is the position vector of the observation point in the reference frame. When dealing with a continuous wave excitation with direction $\hat{k}$, the driving acoustic pressure wave (transducer) can be written as:

$$P(t, \vec{r}) = P_{ac} e^{i(\omega t - \hat{k} \cdot \vec{R})}$$ (3.1)

Using the results from section 2, the scattered pressure from a microbubble is:

$$P(t, \vec{r}_o) = -\frac{\rho \omega^2 R_o^3}{\rho R_o^2} X(\omega) e^{i\psi(\omega, R_o)} P_{ac}$$ (3.2)

$$= \frac{1}{|\vec{R}|} \frac{\omega^2 R_o}{\sqrt{(\omega_o^2 - \omega^2)^2 + (\delta \omega \omega_o)^2}} P_{ac} e^{i(\omega t - |\vec{k} \cdot \vec{R}| + \psi(\omega, R_o))}$$ (3.3)

where $\psi = \arctan \left[ \frac{\delta \omega / \omega_o}{(\omega / \omega_o)^2 - 1} \right]$ is the phase lag associated with a harmonic oscillator. The net pressure wave from a bubble population will be the sum of each individual bubble,
yielding:

\[
P(t, \vec{r}_o) = \sum_i \frac{1}{|\vec{r}_i - \vec{r}_o|} \frac{\omega^2 R_{o,i}}{\sqrt{\left(\omega_{o,i}^2 - \omega^2\right)^2 + (\delta\omega \omega_{o,i})^2}} P_{ac} e^{i\left(\omega t - |\vec{k} \cdot (\vec{r}_i - \vec{r}_o)| + \psi_i(\omega, R_o)\right)} \tag{3.4}
\]

Since \(\vec{r}_o\) is fixed and much bigger than \(\vec{r}_i\), it can be shown by first order Taylor expansion that \(|\vec{r}_o - \vec{r}_i| \approx r_o \left(1 - \frac{k^2}{r_o^2}\right)\). A zero-th order approximation for the decaying term is usually sufficient since its effect is going to be negligible compared to the phase term. Though the phase lag term \(\psi_i(\omega, R)\) depends upon the population size of bubbles, its combined effect with \(|k| |r_o^2 - r_i^2| \gg 2\pi\) will be to give a random phase for each bubble, independently of the bubble size population. For this reason, we will relabel \(\Psi_i = |\vec{k} \cdot (\vec{r}_i - \vec{r}_o)| + \psi_i(\omega, R_o)\) which can be modelled as a random phase \(\in [0, 2\pi]\) according to a flat probability distribution. The equation for the scattered pressure can now be simplified to:

\[
P(t, \vec{r}_o) = \frac{P_{ac}}{r_o} \sum_i A_i(\omega, R_{o,i}) e^{i(\omega t + \Psi_i)} \tag{3.5}
\]

or taking the real part of the previous phasor equation:

\[
P(t, \vec{r}_o) = \frac{P_{ac}}{r_o} \sum_i A_i(\omega, R_{o,i}) \cos(\omega t + \Psi_i) \tag{3.6}
\]

where \(A_i(\omega, R_{o,i}) = \frac{\omega^2 R_{o,i}}{\sqrt{\left(\omega_{o,i}^2 - \omega^2\right)^2 + (\delta\omega \omega_{o,i})^2}}\). Finally using the harmonic addition theorem:

\[
\begin{align*}
P(t, \vec{r}_o) &= \frac{P_{ac}}{r_o} A \cos(\omega t + \Psi) \\
A^2 &= \sum_i \sum_j A_i A_j \cos(\Psi_i - \Psi_j) \\
tan\Psi &= \frac{\sum_i A_i \sin\Psi_i}{\sum_i A_i \cos\Psi_i} \tag{3.7}
\end{align*}
\]

The harmonic theorem guaranties that as long as we add waves of the same frequency, the resulting wave will be a plane wave of the same frequency with modulated amplitude and phase. This equation provides a very fast way to numerically add waves of the same frequency.
3.2.2 Central limit theorem and Rayleigh statistics

It is possible to obtain statistical information (mean, variance) directly from equation 3.8, but it is much simpler to start directly from equation 3.6. In ultrasound imaging, the effect of interference causes a speckle pattern which limits the spatial resolution of the imaging modality. It is moreover well known in the ultrasound community [51, 53] that a speckle pattern formed by a large number of sub-resolution scatterers will behave closely according to Rayleigh statistics. We will now apply this existing theory to the case of a linearized bubble, according to the derivation from [51]. Equation 3.6 is re-expressed as:

\[ P(t, \mathbf{r}_o) = \frac{P_{ac}}{r_o} \left( \cos(\omega t) \sum_i A_i(\omega, R_{o,i}) \cos(\Psi_i) - \sin(\omega t) \sum_i A_i(\omega, R_{o,i}) \sin(\Psi_i) \right) \]

\[ P(t, \mathbf{r}_o) = \frac{P_{ac}}{r_o} \left( \Omega_1 \cos(\omega t) - \Omega_2 \sin(\omega t) \right) \]

\[ P(t, \mathbf{r}_o) = \frac{P_{ac}}{r_o} \sqrt{\Omega_1^2 + \Omega_2^2} \cos(\omega t - \Theta) \] (3.9)

where \( \Omega_1 = \sum_i A_i(\omega, R_{o,i}) \cos(\Psi_i) \), \( \Omega_2 = \sum_i A_i(\omega, R_{o,i}) \sin(\Psi_i) \) and \( \tan \Theta = -\frac{\Omega_2}{\Omega_1} \). In the \( |\mathbf{k} \cdot \mathbf{r} - \mathbf{r}_o| \gg 2\pi \) and large number of microbubble limits, it is possible to apply the central limit theorem. Based upon the central limit theorem, the sum of independent probability distribution functions will always converge toward a Gaussian in the large number limit. In the particular case where each probability function is centred around zero (the present case since \( \langle A_i \cos(\Phi_i) \rangle \approx \langle A_i \sin(\Phi_i) \rangle \approx 0 \) for a random probability function \( \Phi_i \)) the Gaussian will be centered around zero:

\[ P(\Omega_{1,2}) = \frac{1}{\zeta \sqrt{2\pi}} e^{-\Omega_{1,2}^2/(2\zeta^2)} \] (3.10)

The signal to noise ratio of an ensemble of bubbles can be computed using the identity \( \zeta = \sqrt{\frac{1}{2} N \langle A_i^2 \rangle} \) [51] and the well know property that the norm of two zero-centred Gaussian-distributed variables is Rayleigh distributed. Then:

\[ \left\langle |P(\omega, \mathbf{r}_o)| \frac{r_o}{P_{ac}} \right\rangle = \sqrt{\frac{\Omega_1^2 + \Omega_2^2}{2}} = \sqrt{\frac{\pi}{2}} \zeta = \sqrt{\frac{\pi}{4} \sqrt{N} \langle A_i^2(\omega, R_{o,i}) \rangle} \]

\[ \text{Var} \left\langle |P(\omega, \mathbf{r}_o)| \frac{r_o}{P_{ac}} \right\rangle = \frac{4 - \pi}{2} \zeta^2 = \frac{4 - \pi}{4} N \langle A_i^2(\omega, R_{o,i}) \rangle \] (3.11)
3.2.3 Extrapolations to harmonic and wavepackets cases

Equation 3.11 was derived under continuous planar wave and linear oscillator conditions by simplicity, but since only linear operators were used (i.e. additions and recombination), it is possible to push the analysis to non-linear signal propagation using Fourier analysis. This is important since bubbles are non-linear scatterers, and shorter pulses are often desirable to increase the spatial resolution in a B-mode image. Let us assume that our received signal has the following Fourier expansion:

$$P_{\text{scatt}}(t, \vec{r}_o) = P_{ac} \sum_{i,k} A_i(k) \cos(\omega_k t + \psi_i(k))$$  \hspace{1cm} (3.12)

where $A_i(k)$ is the amplitude response for the $i^{th}$ bubble at the $k^{th}$ frequency component. This time, $A_i(k)$ is simply an unknown function that can be found either through simulations or experiment. Re-combining the previous equation yields :

$$P(t, \vec{r}_o) = \frac{P_{ac}}{r_o} \sum_k \left( \cos(\omega_k t) \sum_i A_i(k) \cos(\Psi_i(k)) - \sin(\omega_k t) \sum_i A_i(k) \sin(\Psi_i(k)) \right)$$

$$P(t, \vec{r}_o) = \frac{P_{ac}}{r_o} \sum_k \sqrt{\Omega_1(k)^2 + \Omega_2(k)^2} \cos(\omega_k t - \Theta(k))$$

$$P(t, \vec{r}_o) = \frac{P_{ac}}{r_o} \sum_k P_k(\omega_k) \cos(\omega_k t - \Theta(k))$$  \hspace{1cm} (3.13)

As long as the conditions for the central limit hold, we can apply the same algebra used in the section 3.1.2 to all $\Omega_{1,2}(k)$ pairs separately, and each Fourier amplitude coefficient $P_k(\omega_k)$ will be Rayleigh distributed. As a consequence:

$$\langle |P_k(\omega_k, \vec{r}_o)| \rangle = \sqrt{\frac{\pi}{4}} \sqrt{N} \sqrt{\langle A_i(k)^2 \rangle}$$

$$Var(\langle |P_k(\omega_k, \vec{r}_o)| \rangle) = \frac{4 - \pi}{4} N \langle A_i(k)^2 \rangle$$  \hspace{1cm} (3.14)

This holds both if we deal with broadband responses from short pulses, or non-linear harmonic responses from microbubbles, as long as the central limit theorem condition is satisfied.
3.2.4 Primary properties of Rayleigh statistics

Signal-to-noise ratio

The variance associated to a fully developed speckle pattern is described by equation 3.11. A nice property about Rayleigh distributed functions is that their signal-to-noise ratio is constant \( SNR = \frac{\left< P \right>}{\sigma_P} \approx 1.91 \), independently of the frequency used. Speckle is inherent to the bubble scattered spectrum, limiting our accuracy in determining the resonance frequency and therefore, blood pressure. To compensate for low SNR, stochastic averaging can be used, where many independent measurements are combined to increase the SNR. Two measurements are assumed to be independent if the bubble distribution is sufficiently different such that the two scattered spectra are uncorrelated. In a realistic application, this means that we need to wait a time \( t_{relax} \) in between each measurement during which the bubbles will drag due to the blood flow and redistribute. A more physical condition on the actual relaxation time can be found in Appendix B. The SNR obtained for a certain number of measurements is determined by:

\[
SNR = \sqrt{\frac{\pi}{4 - \pi}} \sqrt{N_{AVE}} \approx 1.9131 \sqrt{N_{AVE}}
\]

(3.15)

Expected mean

A direct consequence of equation 3.14 is that the scattered pressure amplitude does not increase linearly with the number of scattering particles, but increases in proportion to the square root of the number of bubbles. This happens because some of the amplitude is lost by destructive interference. This scattering behaviour from population is already known from red blood cell scattering theories. As the number of scatterers increase, interference effects become progressively important and the acoustic signal per scatterer converges to zero \( \propto \frac{1}{\sqrt{N}} \) in a purely interferential regime. At higher concentration, the driving pulse may get significantly absorbed and/or scattered by the microbubbles, causing additional effects such as shadowing.
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Figure 3.2: Effect of linear and interferential scattering regimes on the mean scattered pressure versus number of scatters. We expect an experimental curve to lie in between these two limits if the central limit theorem is not fully satisfied. (Neglecting shadowing effects which may further decrease the amplitude).

Moreover, the net signal observed is the quadratic mean, or the root mean square, of the scattered pressure of individual bubble $A_i$, and not a weighted average.

3.2.5 K-statistics and validity of the central limit theorem in the portal vein

The use of Rayleigh statistics, although very intuitive, assumes that the number of superposing scatterers is high enough such that the central limit theorem holds (two scatterers are superposed if their point spread functions interfere). Although mathematical formalism requires an infinite number of scatterers, it is well documented in literature that the limit is a good approximation with as few as 10 superposing scatterers [54]. If the number of scatterers is too low such that the central limit theorem breaks, the scattered signal can be modelled using K statistics, a less restrictive model. The properties of the
envelope are determined by the following probability distribution:

\[ p(A) = \frac{2b}{\Gamma(\alpha)} \left( \frac{bA}{2} \right)^{\alpha} K_{\alpha-1}(bA); b = \sqrt{\frac{\alpha}{E[A^2]}}; A \geq 0 \] (3.16)

where \( A \) is the envelope amplitude, \( \Gamma \) is the gamma function, \( K_x \) is the Bessel function of second kind of order \( x \), and \( \alpha \) is related to the number of scatterers \( N_s \) through \( \alpha = (\mu + 1) N_s \) (\( \mu \) is a constant, dependent upon the transducer geometry and the scatterer properties). In particular, Greenleaf [54] has shown that the SNR for a K distribution model follows:

\[ SNR = \frac{m_1}{\sqrt{m_2 - m_1^2}} \] (3.17)

where \( m_1 \) and \( m_2 \) are the first and second moment of \( p(A) \) which can be computed through:

\[ m_\nu = (2\sigma^2)^{\frac{\nu}{2}} \frac{\Gamma(1 + \nu/2) \Gamma(\alpha + \nu/2)}{\alpha^{\nu/2} \Gamma(\alpha)} \] (3.18)

In the case of a spherically focused transducer, a rough approximation of the spatial region over which the bubbles will superpose is a cylinder whose diameter is the FWHM at the focus (\( FWHM \approx 1.4\lambda F \) where \( \lambda \) is the wavelength and \( F \) is the f/# number of the transducer) and whose length is the pulse dimension (\( \sim \lambda \)). In clinical conditions, microbubbles (Definity) are injected with a concentration around 7125 bubbles/ml (value determined using a Coulter counter), which corresponds to about 48 bubbles interfering simultaneously. A simulation of the scattered signal from bubbles using different concentrations is shown in figure 3.3. The low concentration limit is fitted with a K statistics model, while the high limit was fitted with a Rayleigh model. The expected microbubble concentration was indicated with a solid line. It follows that in large blood vessels (such as the portal vein), the Rayleigh limit is a valid simplification. In the case where the size of the blood vessels becomes small, such as a tumour vasculature, the effective volume occupied by blood may be reduced further, and the Rayleigh approximation may become inaccurate.
Figure 3.3: SNR of simulated echo envelope through a less restrictive statistical model (K statistics). The 2 dashed lines correspond to the low and high concentration (Rayleigh) asymptotic limits. The typical microbubble concentration is shown with a solid box (2.25 MHz/3.04 cm aperture spherically focused transducer with SF= 5.08 cm)

### 3.3 Methods

#### 3.3.1 Simulation

The echoes from various bubble populations were computed using the linear response equation (2.15). Spatial populations of microbubbles were simulated by randomly attributing a spatial location to each bubble. An elastic shell model was used to study speckle properties and the effect of radius distribution $P_R$, while the full radius dependent elasticity behaviour was included to quantify the statistical effect of surface tension at rest $P_{\sigma_0}$. All computations were done in *MATLAB*. 
3.3.2 Experiment

All experiments were performed using a single element spherically focused transducer in a pulse echo setup identical to the one described in the previous chapter. To validate the Rayleigh model, the signal-to-noise ratio was measured both for broadband (1-2 cycles) and narrowband pulses (typically 15 cycles). A 2.25 MHz transducer was used for the broadband experiment, while a 3.5 MHz transducer (same as in previous chapter), was used for the narrowband measurements.

The same 3.5 MHz transducer was also used to measure the effect of radius size distribution on the echo. The methods used to control microbubble size distributions will be discussed in the next sections.

3.3.3 Controlling microbubble size distribution

At the moment, there exists no commercially available monodisperse microbubbles. Since the fast production of a bulk volume of microbubbles usually uses sonication or agitation activation, there is very little control over the exact radius distribution obtained. The radius dispersion curves are typically very broad, with coefficients of variation \( \text{CoV} = \frac{\sigma_R}{\mu_R} \) (dimensionless) as large as 100%, and although there exists some repeatability, the variability between two identical vials activated by the same process can be significant. Such statistical variation is inadequate when very accurate and repeatable measurements of the resonance frequency are required.

Mechanical filtration

Mechanical filtration can be used to reduce further the CoV of sonicated microbubbles. This technique is relatively fast and inexpensive since the microbubble solution simply needs to diffuse through porous filters (available commercially), but suffers from major drawbacks. Filtering can only remove bigger bubbles than the size of its constitutive
micropores; it is a low-pass size filter, but not bandpass. Microbubbles are also compressible much more than cells or solid particles and can tunnel through small pores, reducing further the efficiency of the technique. An example of mechanically filtered microbubbles is shown in figure 3.4. The bubbles much larger than the pore size (indicated by a black line) will be removed by the filter while bubbles with size similar to the pores have a finite probability to tunnel.

Figure 3.4: Mechanical filtering of microbubbles using a porous filter (1.2µm pores size, indicated by a vertical thick dashed line) of Borden phospholipid microbubbles (unfiltered in blue dashed, filtered in solid green).

Centrifugal filtration; decantation

Feshitan et al. [55] have described a method to isolate target microbubble sizes by using a centrifuge. The technique, similar to decantation, exploits the simple idea that bubbles slowly float under the effect of gravity, and that bigger bubbles float faster. Centrifuging speeds up the process to a couple of minutes instead of hours for decantation floatation. To extract microbubbles of a particular target size, two successive centrifugations are required:
The first centrifugation is slow and serves to remove the bigger bubbles.

A faster centrifugation of the supernatant from step 1 isolates the bigger bubbles.

The slowly rising motions of bubbles in water can be accurately described by Stokes flow theory (low Reynolds number model, or viscosity dominant regime). The buoyancy velocity $u_b$ can then be estimated using:

$$u_b = \frac{2(\rho_w - \rho_b)}{9\eta_w} R^2 g_c$$

where $\rho_w$ is mass density of water, $\eta_w$ is the dynamic viscosity of water, $R$ is the bubble’s radius and $g_c$ is the centrifugal acceleration. An experimental demonstration of selective centrifuge filtration on microbubbles is shown in figure 3.5. Centrifuge filtration is much more efficient to remove bigger bubbles than smaller bubbles since the second filtration consists more of an enrichment process than an effective filtration. Additional iterations can be used to further decrease the CoV, at the cost of reduced microbubble concentration.

### 3.4 Results

#### 3.4.1 Simulation

Signal-to-noise ratio

The linearized scattering from a population of 1000 identical (where Rayleigh statistics should apply) 3.0 µm radius microbubbles to a continuous wave excitation was computed using equation 3.8 directly (i.e., not using the central limit theorem). The position of each bubble was determined through random allocation, and the process was repeated 100 times. The agreement between the central limit theorem SNR and the simulated SNR was good, within 4% error. This result was independent of the frequency used.
Figure 3.5: Size population $P(R)$ measured with a Coulter counter for centrifuge filtered (green) and native sonicated (red) microbubbles.

Figure 3.6: Linearized simulation for a 3 $\mu m$ radius microbubble population response. The pressure response is shown at left while the inverse SNR is displayed at right. Stochastic averaging reduces the effect of speckle.

Deviations observed are most likely due to the finite number of distributions generated (results are displayed in figure 3.6).

This confirms that in the fully developed speckle limit, each independent Fourier
coefficient of the scattered pressure pulse will behave according to Rayleigh statistics. Therefore the SNR of a measurement will depend only upon the number of independent spectra compounded, imposing a temporal resolution limit on our blood pressure measurements.

**Effect of size distribution**

We will now suppose that $N_{AVE}$ is sufficiently large such that we can study the effect of a bubble size population, independently of interference. According to equation 3.14, the expected pressure amplitude for the $k^{th}$ harmonic is:

$$\langle |P_k(\omega_k, \vec{r}_o)| \rangle = \sqrt{\frac{\pi}{4}} \sqrt{N} \sqrt{\langle A_i(k)^2 \rangle} \quad (3.20)$$

The net signal observed is the quadratic mean, or the root mean square, of the scattered pressure of individual bubble $A_i$ (different size). A weighted average $< A_i(k) >$ might have been expected instead, but while the pressure field itself is an additive quantity, the backscattered amplitude is not. To study the effect of radius size distribution, we will assume the bubble is away from buckling such that the elasticity modulus $\chi$ is roughly constant.

The linear echos from a population of 1000 microbubbles following a Gaussian radius distribution centred around 2, 4 and 6 \(\mu m\) were calculated for increasing values of standard deviations. The backscattered spectra, the artificial shift in the resonance frequency and the decrease in the quality factor are shown in figure 3.7. The CoV ($CoV = \frac{\sigma R}{\mu R}$, dimensionless) was also introduced to normalize and quantify the spread of the microbubble size distributions.

Two dominant effects were observed:

- **Shift in the resonance frequency**: The resonance frequency of a microbubble is specific to its radius. The resonance frequency will decrease with an increase of the microbubble radius. In addition, bigger bubbles emit stronger echoes than smaller
Figure 3.7: Effect of the bubble size distribution on the scattered signal by changing the standard variation of a Gaussian population size distribution with 3.0 \( \mu m \) mean radius. The shift of resonance frequency (bottom left) and the decrease of the quality factor (bottom right) were computed from the scattered pressure (top) for microbubble populations of increasing CoV and various mean radii.

bubbles. This distorts the resonance peak and shifts the resonance frequency as a population becomes more polydisperse. Shifts in the resonance frequency cause systematic errors in static pressure measurements.

- **Reduction of quality factor** \( Q \): The RMS of a bubble population echo spectrum is smeared by compounding peaks of different centre frequencies, which artificially
decreases the quality factor ($Q = \frac{f}{\text{FWHM}_f}$) of the resonance peak. A less defined resonance peak causes an increase in the random error of the resonance frequency measurement, therefore reducing our accuracy in measuring pressure.

The $Q$ factor decreases particularly fast, as the CoV of the bubble population is increased above 3% and shifts in the resonance frequency are observed when $CoV > 10\%$. Such effects are undesired because they greatly reduce our accuracy in detecting the resonance frequency, and therefore our ability to determine the gauge blood pressure. A CoV of $\sim 3\%$ should be targeted, based on these simulation results.

**Effect of variation of $\sigma_o$**

It has been observed by Sijl [3] that two bubbles of identical size may respond very differently to the same acoustic pulse depending upon the concentration of the surfactant in their shell, which affects directly the shell surface tension. A natural statistical consequence is that even monodisperse microbubbles will have different surface tensions, and will therefore buckle at slightly different radii. In the Sijl model, the surface tension at rest $\sigma_o(R_o)$ is related to the buckling radius $R_{\text{buckling}}$ through a one-to-one function, meaning that it is possible to compute $R_{\text{buckling}}$ from a measurement of $\sigma_o(R_o)$, and vice-versa. In this section, we will work using the formalism of the probability distribution of $R_{\text{buckling}}$.

A very intuitive understanding of the effect of $R_{\text{buckling}}$ on the resonance frequency versus blood pressure curve can be obtained through figure 3.8. A microbubble undergoes a steep reduction of its resonance frequency as its radius approaches its buckling radius. If the buckling radius is reduced, higher static pressure will be required to push the microbubble to its buckled state. Ideally, controlling the buckling radius of a microbubble provides a very efficient method to specify the pressure limits between which a microbubble will be a good manometer.
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Figure 3.8: Parameter study of the effect of $R_{\text{buckling}}$ on the resonance frequency versus blood pressure inversion curve. The bubble had a 3.0 $\mu m$ radius at rest.

It is expected that within a population, the concentration of the surfactant will vary for each bubble and therefore we should expect each of them to have a slightly different buckling radius. Although it is straightforward to compute the linear response (equation 2.15) of bubbles with various buckling radii, the extent of the statistical fluctuations of the buckling radius within a bubble population remains to be quantified experimentally and further assumptions are required. We will assume all microbubbles start within the elastic regime ($R_o > R_{\text{buckling}}$) and that $R_{\text{buckling}}$ is distributed according to Gaussian statistics ($P_{R_{\text{buckling}}} \propto e^{-\frac{(R - \mu)^2}{2\sigma^2}}$). Only two extremes cases will be studied: one where all bubbles have about the same initial surface tension ($\sigma_R = 0.0001 \mu m$) and one where all bubbles have different initial surface tension ($\sigma_R = 1 \mu m$) (figure 3.9).

While increasing $\sigma_R$ (buckling radius variance) does not cause artifactual shifts in the resonance frequency as observed for increasing bubble size distribution, it reduces the steepness within the transition regime (reducing our resolution in pressure measurement).
Figure 3.9: Effect of statistical spread of $R_{\text{buckling}}$ on the resonance frequency versus blood pressure inversion curve. Each bubble had a 3.0 $\mu m$ radius at rest, but a buckling radius varying according to a probability distribution. At left, each bubble is assumed to have the same buckling radius while at right a different buckling radius was attributed to each microbubble.

### 3.4.2 Experiment

**Signal-to-noise ratio**

Our previous analysis suggests that the formula for SNR should apply to each frequency component of a wavepacket signal or even harmonic emission (this is also assuming that the concentration of bubbles is high enough such that the interference pattern is fully developed). For this purpose, a broadband pulse echo experiment was conducted for freshly activated samples of *Definity* microbubbles, a commercially available phospholipid shell ultrasound contrast agent, using a similar setup to the one shown in the previous chapter. The bubbles were insonified using a one cycle pulse at the centre frequency of a 2.25 MHz high precision spherically focused transducer. The experiment was repeated 100 times such that statistical analysis could be applied. The bubble concentration was estimated to 7125 bubbles/ml (or $\sim 48$ interfering bubbles per focal volume) using a Coulter Counter apparatus. The SNR was computed directly from the Fourier transform (fre-
frequency component) of the ensemble of backscattered echoes and is shown in figure 3.10. In agreement with theory, each Fourier coefficient followed Rayleigh statistics within 5% error.

Figure 3.10: Comparison between the expected SNR using the central limit theorem (red) and the experimental SNR of freshly activated Definity using a broadband pulse (blue). 100 pulse echo measurements were conducted to get a significant statistical set.

A similar measurement was also conducted using a sequence of narrowband pulses. The SNR obtained was similar to the broadband measurement, although sometimes small deviations for the second harmonic (9%) were observed, most likely attributed due to post-processing methods (envelope detection, filtering, etc.). Results are displayed in figure 3.11. The frequency range for the second harmonic is smaller than the fundamental due to the transducer’s finite bandwidth (the second harmonic will be detected at twice the insonification frequency).
Figure 3.11: Measurement of the SNR from a centrifuge-filtered phospholipid microbubble population (discussed in next section) using a narrowband pulse sequence for both the fundamental (left) and the second harmonic (right). The experimental curve (blue) is compared to the Rayleigh statistics prediction (red).

Effect of size distribution: Spectra of broad and narrow centrifuge size-filtered bubbles

To demonstrate experimentally the effect of bubble size population, the backscattered echo of unfiltered (phospholipid bubbles made through sonification) and centrifuge-filtered bubbles were compared through pulse-echo measurements. The scattered signal was measured at the focus of the transducer and the frequency dependence was compensated using the calibration echo from the quartz plate.

To account for speckle SNR, stochastic averaging was used with about 100 independent measurements ($SNR \approx 1.91\sqrt{100} \approx 19.1$). Speckle is not white noise and therefore if one would measurement twice the exact distribution of scatterers, one would get exactly the same speckle pattern. The speckle-independent condition was achieved by letting the bubbles flow in and out of the acoustic chamber continuously and waiting about 200-250 ms between each measurement. Three millisecond delays were added in between each narrowband pulse to avoid transient behaviour due to previous pulses. Such time delays
also minimized the overheating from the amplifier.

Figure 3.12: Microbubble backscattered response $H_B$ (first and second harmonic) for both centrifuge-filtered (green) and native (red) size populations. The size population $P(R)$ measured with a Coulter counter is also shown.

Figure 3.12 compares the bubble transfer function $H_B$ (first and 2nd harmonic) for both a centrifuge-filtered sample and an unfiltered sample at low acoustic pressure (to avoid transducer nonlinearities and bubble buckling). The dispersion relation $P(R)$ was obtained by using a Coulter Counter and was measured just before conducting the experiment to reduce the systematic error related to the diffusion of the gas core through the microbubble lipid shell. As expected from the simulations, the centrifuge filtered bubbles have a higher quality factor $Q$ and resonance frequency than the initial population. While we can hardly determine the resonance frequency for the initial bubbles, the resonance peak is clearly visible for the centrifuged bubbles. A shift of the resonance frequency from 2.3 MHz to 3.8 MHz was observed (40 % change). The CoV of the centrifuged microbubbles (≈ 0.7) is still high enough such that if the CoV were decreased further, additional shifts in the resonance frequency and increases of $Q$ would be measured.
3.5 Conclusion and discussion

The purpose of this chapter was to gain insight regarding the reproducibility of measuring blood pressure using phospholipid microbubbles’ resonance frequencies, in particular within the portal vein. Three statistical effects in particular were studied: spatial distribution, radius size distribution and variation of the surfactant concentration. Spatial distribution causes a fluctuating speckle pattern with primary properties following Rayleigh statistics in the case of large vessels (such as the portal vein). This was verified both in simulation and in an experiment mimicking clinical conditions. The maximum SNR per spectrum acquisition is $\sim 1.91$, virtually too low for accurate resonance frequency measurement, and temporal compounding must be used to increase the SNR, reducing our temporal resolution. Increasing the spread of bubble size distribution (neglecting buckling) causes a shift of the resonance frequency and a reduction of the resonance peak sharpness, introducing both a systematic and random error in the method. These effects can be avoided by using populations with a coefficient of variation in radius smaller than 3\%. Finally, microbubbles with different surfactant concentrations will buckle at different values of blood pressure, reducing the steepness of the variation of the resonance frequency within the transition regime for populations of microbubbles.
Chapter 4

Conclusions

4.1 Summary

The objective of this thesis was to determine whether or not measuring blood pressure using microbubbles combined with ultrasound is feasible. Such a non-invasive technique would be appropriate, in particular, for patients presenting portal vein hyper-pressure symptoms. Fairbank et al. [19] as early as 1977 demonstrated that blood pressure affects unshelled microbubbles in such a way that a precise measurement of the resonance frequency of microbubbles should allow us to recover the local gauge blood pressure, though the effects predicted were relatively small for clinical applications and unshelled microbubbles had a very short half-life. Nowadays microbubbles are shelled (protein, polymer, phospholipid, etc.), considerably increasing their half-life. Based upon the recent observation that the sub-harmonic backscattered echo amplitude of phospholipid-coated microbubbles is strongly dependent upon blood pressure due to the buckling behaviour of microbubbles, we proposed that buckling of phospholipid microbubbles should also cause strong shifts, bigger than the ones predicted by Fairbank et al. [19], in the resonance frequency of microbubbles.

Both the De Jong and the Marmottant (buckling surface tension) equations of motion

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were used to model the radial behaviour of protein shelled and phospholipid shelled microbubbles, respectively. Those models were described in chapter 2, and linearization was applied to measure analytically the first order effect of blood pressure on these two shell models. It was shown using this approach that viscoelastic microbubble are about as sensitive to blood pressure as unshelled microbubbles, while phospholipid microbubbles, within the transition regime, are roughly 40-120 times more sensitive to static fluid pressure changes, because \( \chi \) varies very fast from \( \sim 0.54 N/m \) to \( \sim 0.0 N/m \) within this regime.

Based upon these results, thorough simulations of the equations of state were conducted for both types of microbubbles using sequences of narrowband pulses for increasing values of driving acoustic pressure. Protein-shelled microbubbles behave very similarly to their linearized counterparts, while important finite size oscillation effects were seen for phospholipid microbubbles. As driving pressure is increased, the microbubble starts to experience a fraction of its oscillation cycle either in the buckled or free state (both where the surface tension and elasticity \( \chi \) are zero) and causes a steep decrease of the resonance frequency. This suggests that the acoustic pressure must remain low in order for phospholipid microbubbles to be good manometers. To validate our simulations, experiments were conducted using a pulse-echo \textit{in vitro} setup. A narrowband pulse-echo sequence was used and the resonance frequency was measured against hydrostatic pressure for both protein (Optison) and phospholipid (Borden recipe, native and size filtered) microbubbles. A significant drop of the resonant frequency versus \( P_o \) (0.20 MHz and 0.15 MHz for narrowband and broadband radius distributions respectively) over as few as 10 mmHg was measured for phospholipid microbubbles while no significant changes were observed for protein shells over a clinical range of blood pressure, in agreement with the simulations.

The current problem with this approach is reproducibility of the measurement between different bubble populations and a large experimental error associated with a low
resonant peak quality factor $Q$. For this purpose, chapter 3 tackled the problem of scattering from microbubble populations. In relatively large blood vessels such as portal vein, the microbubble concentration is such that the demodulated signal amplitude is dominated by Rayleigh statistics. As a direct consequence, each harmonic amplitude will be limited to a low signal-to-noise ratio ($\sim 1.91$), independently of frequency, which can be increased by compounding many statistically independent measurements. Statistical variation of the microbubbles’ physical properties such as their radii size distribution and shell properties will also affect the populations’ echoes. A systematic increase of the CoV (coefficient of variation) of microbubble’s radii size distribution was found to cause both a shift in the resonance frequency and a decrease in the quality factor. The required CoVs necessary to avoid such artifacts are 10% and 3%, respectively. Finally, using microbubbles with different surfactant concentrations causes a spread in the buckling radius $R_{\text{buckling}}$ within a population. This was found, through simulation, to affect the steepness of variations of the resonance frequency within the transition regime.

4.2 Discussion

While it is true that blood pressure compression will change the microbubble size, and therefore the geometric scattering cross-section, a 10 mmHg variation of blood pressure, the required resolution for portal vein pressure measurement, will typically compress microbubble by 6-20 nanometers. For this reason, simpler microbubbles, such as unshelled and protein shelled bubbles, will respond only very weakly to blood pressure. Other types of microbubbles though will respond strongly to blood pressure because of their shell properties. In particular, it was shown in this thesis that phospholipid shell microbubbles undergo a phase transition (buckling) if the blood pressure is increased to the point that the surfactant density on the bubble’s surface reaches maximal concentration. The symmetry of the shell is then broken, and the elasticity of the bubble quickly drops
to zero, increasing the sensitivity of the microbubble to blood pressure by 40-120 times, compared to the response of an unshelled microbubble. Phospholipid microbubbles are therefore sensitive enough to measure 10 mmHg pressure variations through measurement of their resonance frequency.

Moreover, in order to get reproducible measurements of blood pressure, some statistical conditions need to be met:

- Measurements need to be compounded such that the signal-to-noise ratio is high enough ($SNR \approx 1.91\sqrt{N_{AVE}}$). This is at the cost of either temporal or spatial resolution.

- Microbubbles need to be of homogeneous radii. In particular, the CoV of the radius needs to be smaller than 3%.

- The concentration of the phospholipid surfactant (microbubble shell) needs to be homogeneous within a population such that microbubbles will buckle at the same radius.

Although it is straightforward to design a clinical system that will satisfy the required SNR conditions, clinically available microbubble contrast agent are mainly produced through sonication, a poorly reproducible method, which results in a highly variable size distribution ($CoV \gg 10\%$). Centrifuging the microbubbles, although it reduces the CoV, is not selective enough to reach the 3% threshold. The particular mechanical process used to generate microbubbles, dictacting the ultimate concentration of lipid on the surface, also suggests a very variable surfactant shell concentration (this variability has yet to be measured experimentally).

Fortunately, it has been recently demonstrated [4] that a 3-inlet microfluidic system can be used to produce almost monodisperse microbubbles. Perfluocarbon (PFC) gas is injected at high throughput in the central channel and the aqueous shell solution is injected through both upper and lower channels. The microfluidic microbubbles are
formed in a small nozzle at the inlets’ intersection, where both immiscible phases mix. Depending on the flow rate and size of the nozzle used, the bubbles will have different diameters and CoVs. According to figure 4.1 (from Seo et al. [4]), the obtained CoV are always smaller than 3%. Since the microfluidic environment is well controlled, we may surmise that each bubble will have a more similar surfactant concentration.

Using these phospholipid shell microbubbles made through a microfluidic system, a reproducible blood pressure measurement with a resolution as small as 10 mmHg should be feasible.

Figure 4.1: Microscope image of the 3-inlet microfluidic devices used to produces microfluidic microbubbles. Different flow rates produce bubbles of different size and CoV. Figure from Seo et al. [4]
4.3 Future Work

4.3.1 Ultrafast optical imaging of the effect of blood pressure on bubble radial response

In this thesis, we have inferred buckling based upon various evidences present in the backscattered echoes from a population. While this seems reasonable considering observations from previous groups (namely Marmottant \textit{et al.} [24] and Shi \textit{et al.} [27]), a systematic optical validation of the effect of static fluid pressure on buckling has, to our knowledge, yet to be done (buckling within finite size oscillation has been observed by Sijl [3]). To get a resolved image of a buckled bubble, the camera’s shutter must remain opened only within a fraction of the bubble oscillation \( < 0.1 \mu s \) at a repetition rate of 10 to 20 MHz. While it is possible to use fluoroscopy to get sparse stroboscopic images, the radial response \( R(t) \) of a buckling microbubble to a pulse can only be achieved by using a turbine shutter camera, such as the Brandaris camera at Erasmus (Netherlands). From the \( R(t) \) data, it could be possible to quantify some interesting properties such as the fractional part of oscillation within which the bubble is buckled.

4.3.2 Experimental characterization of buckling radius for microbubble populations

While it is straightforward to measure the variation in the bubble radius by using a Coulter Counter, it is much more difficult to measure \( P(\chi_o) \) and \( P(\sigma(R_o)) \). A measurement of \( P(\chi_o) \), i.e., the elasticity of the layer, can be done optically, or acoustically using a single microbubble setup, similarly to the method we used in this thesis to measure blood pressure. By measuring single microbubble responses to narrowband excitations at different frequencies, it is possible to extract elasticity and damping parameters by fitting the following equation to each individual resonance peak:
Chapter 4. Conclusions

\[
X(\omega) = \frac{1}{\rho R_o^2} \frac{P(\omega)}{\sqrt{\left(\omega_o^2 - \omega^2\right)^2 + \left(\delta \omega \omega_o\right)^2}} \tag{4.1}
\]

\[
w_o = \sqrt{\frac{1}{\rho R_o^2} \left(3\kappa P_o + \frac{2(3\kappa - 1)\sigma}{R_o} + \frac{4\chi(R_o)}{R_o}\right)}
\]

\[
\omega_{res,R} = \frac{1}{\sqrt{1 - \delta^2/2}} \omega_o
\]

This approach has been done by Van der Meer [49] optically for bubbles within the elastic regime.

A relatively simple method to measure \(\sigma(R_o)\), including the buckling effects, was recently proposed by Sijl [3]. Using weakly non-linear analysis, it can be shown that if \(R = R_o (1 + A_0 + A_1 \sin(\omega t) + A_2 \sin(\omega t))\), the compression/expansion only correction to the equilibrium radius \(A_o\) is related to \(A_1^2\) through:

\[
A_0 = \frac{A_1^2}{2\omega_o^2} \left(\frac{9P_o \kappa (\kappa + 1)}{2R_o^2 \rho} - \frac{\xi(A_1)}{R_o^3 \rho} - \frac{1}{2} \omega_o^2\right) \tag{4.2}
\]

where \(A_o\) can be found by using a low-pass filter on the \(R(t)\) data. An example of this technique is shown in figure 4.2.

By repeating this procedure on a statistically significant number of microbubbles from a population, it could be possible to recover insight about the \(P(\chi_o)\) and \(P(\sigma(R_o))\) functions, and therefore understand better the variation of the concentration of phospholipid surfactant within populations.

### 4.3.3 The effect of microbubble deflation

Another concept which has not been tackled in this thesis is the stability of microbubbles over time. In particular, it was shown experimentally by Marmottant et al. [24] that bubbles at rest, even in a saturated atmosphere, will deflate quickly after being created, but will become much more stable as the buckling radius is reached. Gas diffusion outward from the microbubble happens because the gas pressure inside of the bubble
Figure 4.2: Experimental method from Sijl to evaluate the surface tension at equilibrium. $A_0$ and $A_1$ are the amplitude of the 0th and 1st harmonic respectively. Data and figure from Sijl [3]

is always higher than the partial gas pressure of the surrounding liquid due to surface tension (a quantitative formulation of this phenomenon was given by Plesset and Epstein [36]). It is therefore expected that within an old population (old could refer to matters of minutes), bubbles are expected to shrink very close to their buckling radius, no matter their initial size. This could explain why even broad size distributions of microbubbles buckle at low static pressure (as observed in this thesis) and could potentially provide a way to control the buckling radius.
Appendix A

Linearized solution of the Rayleigh-Plesset equation

A.1 Evaluation of the linearized response

The motion of microbubbles in an ultrasound beam can be simulated using the Rayleigh-Plesset equation. For shelled microbubbles, the Marmottant equation can be used (first order expansion of the surface tension):

$$\rho \left( R R'' + \frac{3}{2} R'^2 \right) = \left( P_0 + \frac{2\sigma}{R_0} \right) \left( \frac{R}{R_0} \right)^{-3\gamma} - P_0 \frac{2\sigma}{R} - 4\chi \left( \frac{1}{R_0} - \frac{1}{R} \right) - \frac{4\mu}{R} \frac{R'}{R} - \frac{4\kappa_o R'}{R^2} - P(t)$$

(A.1)

For very small excitations, linearization yields:

$$R = R_0 (1 + x)$$

(A.2)

$$R' = R_0 x'$$

(A.3)

$$R'' = R_0 x''$$

(A.4)

where $x \ll 1$ and is non-dimensional. Substituting these expressions in the Rayleigh-
Appendix A. Linearized solution of the Rayleigh-Plesset equation

The Rayleigh-Plesset equation gives:

\[
\rho \left( R_o^2 (1 + x) x'' + \frac{3}{2} R_o^2 x'^2 \right) = \left( P_o + \frac{2\sigma}{R_o} \right) (1 + x)^{-3\gamma} - P_o - \frac{2\sigma}{R_o(1+x)} \tag{A.5}
\]

\[-4\chi \left( \frac{1}{R_o} - \frac{1}{R_o(1+x)} \right) - \frac{4\mu x'}{R_o^2 (1+x)} - \frac{4\kappa_s R_o x'}{R_o^2 (1+x)^2} - P(t) \tag{A.6}
\]

\[
\rho R_o^2 x'' = \left( P_o + \frac{2\sigma}{R_o} \right) (1 - 3\gamma x) - P_o - \frac{2\sigma}{R_o} (1 - x) - \frac{4\chi}{R_o} x - 4\mu x' - \frac{4\kappa_s x'}{R_o} - P(t) \tag{A.7}
\]

\[
\rho R_o^2 x'' = - \left( P_o + \frac{2\sigma}{R_o} \right) (3\gamma x) + \frac{2\sigma}{R_o} x - \frac{4\chi}{R_o} x - 4\mu x' - \frac{4\kappa_s x'}{R_o} - P(t) \tag{A.8}
\]

\[
x'' + \left[ \frac{4\mu}{\rho R_o^2} + \frac{4\kappa_s}{\rho R_o^3} \right] x' + \frac{1}{\rho R_o^2} \left[ (3\gamma) \left( P_o + \frac{2\sigma}{R_o} \right) - \frac{2\sigma}{R_o} + \frac{4\chi}{R_o} \right] x = -\frac{P(t)}{\rho R_o^2} \tag{A.9}
\]

\[
x'' + \omega_o \left[ \frac{4\mu}{\rho R_o^2 \omega_o} + \frac{4\kappa_s}{\rho R_o^3 \omega_o} \right] x' + \frac{1}{\rho R_o^2} \left[ (3\gamma) \left( P_o + \frac{2\sigma}{R_o} \right) - \frac{2\sigma}{R_o} + \frac{4\chi}{R_o} \right] x = -\frac{P(t)}{\rho R_o^2} \tag{A.10}
\]

This is the equation of a damped linear oscillator. Comparing with the standard form of a damped driven harmonic oscillator \(x'' + \omega_o \delta x' + \omega_o^2 x = -\frac{P(t)}{\rho R_o^2}\), we identify by association:

\[
\omega_o = \sqrt{\frac{1}{\rho R_o^2} \left( 3\gamma P_o + \frac{2(3\gamma - 1)\sigma}{R_o} + \frac{4\chi}{R_o} \right)} \tag{A.11}
\]

\[
\delta = \frac{4\mu}{R_o^2 \rho \omega_o} + \frac{4\kappa_s}{R_o^3 \rho \omega_o} \tag{A.12}
\]

where \(\omega_o\) and \(\delta\) are respectively the nature resonance frequency and the damping of the system. The spectral response of the linear oscillator is then found by taking the Fourier transform of the driven and damped harmonic oscillator differential equation such that:

\[
\int \left[ x(\omega) \left( -\omega^2 + i\omega_0 \omega \delta + \omega_o^2 \right) + \frac{P(\omega)}{\rho R_o^2} \right] e^{i\omega t} dt = 0 \tag{A.13}
\]

\[
x(\omega) \left( -\omega^2 + i\omega_0 \omega \delta + \omega_o^2 \right) + \frac{P(\omega)}{\rho R_o^2} = 0 \tag{A.14}
\]

\[
x(\omega) = \frac{1}{\rho R_o^2 \left( -\omega^2 + i\omega_0 \omega \delta + \omega_o^2 \right)} \tag{A.15}
\]
Taking the amplitude and phase of the complex response:

$$|x(\omega)| = \frac{1}{\rho_R^2} \frac{P(\omega)}{\sqrt{(\omega^2 - \omega_o^2)^2 + (\omega_o \delta)^2}}$$  \hspace{1cm} (A.16)

$$\psi = \arctan \left[ \frac{\delta \omega / \omega_o}{(\omega / \omega_o)^2 - 1} \right]$$  \hspace{1cm} (A.17)

### A.2 Determination of the resonance frequency

The resonance frequency is defined mathematically by the condition \( \frac{d|X(\omega)|}{d\omega} = 0 \) such that:

$$\frac{dx(\omega)}{d\omega} = 0$$  \hspace{1cm} (A.18)

$$\frac{1}{2} \frac{4 (\omega^2 - \omega_o^2) \omega + 2 \delta^2 \omega_o^2 \omega}{(\omega^2 - \omega_o^2)^2 + (\omega_o \delta)^2} = 0$$  \hspace{1cm} (A.19)

$$\omega^2 = \omega_o^2 - \frac{\delta^2 \omega_o^2}{2}$$  \hspace{1cm} (A.20)

$$\omega_{RES,R} = \omega_o \sqrt{1 - \frac{\delta^2}{2}}$$  \hspace{1cm} (A.21)

$$f_{RES,R} = f_o \sqrt{1 - \frac{\delta^2}{2}}$$  \hspace{1cm} (A.22)

Where the R subscript indicates that the resonance occur for the radial motion of the bubble only. A similar expression for the resonance of the scattered pressure can also be obtained similarly by using \( P_{\text{scatt}} \approx \frac{1}{2} \rho R_o^2 \dot{R} \) which becomes exact in the linear limit. This yields:

$$\frac{d (\omega^2 |x(\omega)|)}{d\omega} = 0$$  \hspace{1cm} (A.23)

$$\frac{d}{d\omega} \left( \frac{1}{\sqrt{(1 - \omega^2 / \omega_o^2)^2 + (\omega_o \delta / \omega)^2}} \right) = 0$$  \hspace{1cm} (A.24)

$$\omega_o^2 \left( 1 - \frac{\omega^2}{\omega_o^2} \right) - \frac{1}{2} \omega_o^2 \delta^2 = 0$$  \hspace{1cm} (A.25)

$$f_{RES,P} = f_o \frac{1}{\sqrt{1 - \delta^2/2}}$$  \hspace{1cm} (A.26)
Appendix B

Time scale for speckle pattern decorrelation

In our current pulse-echo experimental setup, a measurement requires about 1 minute ($\approx 0.02\,Hz$) for a full spectral acquisition with sufficient SNR, the main limitation being associated to the 200-250 ms delays in between each measurement (attributed to the cool-down time of the amplifier). Although this delay is long enough such that measurements are fully decorrelated, a target temporal resolution would be about 10 Hz such that diastolic and systolic pressure can be clearly identified.

A better physical understanding of the decorrelation time scale can be obtained using phasors (the amplitude of the phasor is the amplitude of the wave and the phase is the angle with the real axis). When dealing with a multi-wave problem (of same frequency), the net wave will simply be the vectorial sum of all phasors, and the complex problem of the scattering from an ensemble of bubbles is then compacted to a 2D vector plot. When doing measurements on different bubble populations, both the amplitude of the vector (depending of the position in the ultrasound beam and the size of the bubble insonified) and the phase (related to changes in flight path) are affected. The phasors will then shrinks/grow and rotate around the origin respectively, and two measurements
will be independent if those changes are statistically significant. This will be the case in particular if enough time is left for the blood flow to refresh the bubble population. The corresponding time scale will then be associated to the diffusion time of a bubble through the ultrasound A-mode beam. Approximating the width of the A-mode $\delta$ to 0.39 mm and the blood flow $v_{\text{blood}}$ to 1 cm and 10 cm, the obtained diffusion times are 39 ms and 3.9 ms respectively.

**Acquisition #1**
(time $= 0$ ms)

**Acquisition #2**
(time $\approx 200$ ms)

Figure B.1: The bubble flows between each measurement ($\approx 200$ms delays). Two measurements are independent if the time delay is large enough such that spatial distributions are uncorrelated.

This is a sufficient but not necessary condition. From Doppler ultrasound, we know that very small changes in phase can be measured since the wavelength of sound in water for clinical frequencies is on the order of hundreds of microns. Therefore there must exist another time scale, the correlation time $t_{\text{corr}}$ of the speckle pattern, which will be intrinsic to the decorrelation length. For short time interval, the change of phase for a
Appendix B. Time scale for speckle pattern decorrelation

A bubble due to diffusion will be:

$$\Delta \theta_1 = k (|x_1| - |x_1 + \vec{v}_{\text{blood}}t|)$$

$$\Delta \theta_1 \approx k \vec{v}_{\text{blood}} \cdot \vec{u}_{\text{perp}}t$$  \hspace{1cm} (B.1)

where $\Delta \theta$ is the phase change, $x_1$ is the position of the bubble, $t$ is the elapsed time and $\vec{u}_{\text{perp}}$ is the vector normal to the ultrasound transducer. The time required for a bubble to complete a full $2\pi$ rotation can be shown to be in hundreds of microseconds for blood flow conditions, about the pulse repetition rate of a scanner.

It is not sufficient to change the phase of all microbubbles (the absolute phase) to cause the speckle pattern to decorrelate. Indeed it is illustrated in figure B.2 that a constant phase offset identical for all microbubbles will only make the net phasor rotate, leaving the amplitude unaffected. Instead, the speckle pattern will decorrelate if and only if be the relative phase in between the various bubbles $\Delta \theta_1 - \Delta \theta_2$ is modified (i.e. the bubbles phasors will rotate at different rates). The correlation time will then not only be associated to the local flow properties, but to the variance of the flow within the different regions of interest. For instance, laminar flows (parabolic velocity profiles) will have a shorter decorrelation time than fully inviscid non-turbulent flows (flat velocity profile). The correlation time will also be dependent upon the orientation of the transducer.

Therefore, there exists two characteristic times: the diffusion time ($t_{\text{diff}}$) and the correlation time ($t_{\text{corr}}$). $t_{\text{diff}}$ is associated to a complete refreshment of a bubble population within a single A-line while $t_{\text{corr}}$ corresponds to the decorrelation of the different phasors within a population. In most cases, where each bubble will experience a different velocity, $t_{\text{corr}}$ is much smaller than $t_{\text{diff}}$ and is the characteristic time scale of the system (in the order of $\sim 100\mu s$). This seems to suggest that a 10Hz blood pressure measurement repetition rate should be feasible.
Figure B.2: Schematic illustration of the speckle pattern decorrelation process. Blue and orange correspond to 2 different measurements at different times. The thin arrows are signal from individual bubbles (2 shown here) and the thick one is the net sum. If all bubbles are shifted by an identical phase (A), the net sum is only rotated leaving the net amplitude unaffected. Decorrelation happens if bubbles are shifted by different phase offsets (B).
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