Acoustic and cardiovascular quantification by transesophageal echography and Luminity(R) contrast agent dilution

Hermens, B.P.G.

Award date:
2007
Acoustic and cardiovascular quantification by trans-esophageal echography and Luminity® contrast agent dilution

By: B.P.G. Hermens

M. Sc. Theses
Project period: October 2006 / October 2007
Report number: 23-07

Supervisors:
Prof. Dr. H.H.M. Korsten (Catharina Hospital)
Dr. Ir. M. Mischi (Eindhoven University of Technology)

In cooperation with:
CATHARINA-ZIEKENHUIS
PHILIPS
Bristol-Myers Squibb

The Department of Electrical Engineering of the Eindhoven University of Technology accepts no responsibility for the contents of M.Sc. theses or practical training reports
Summary

Cardiac quantification nowadays still requires the employment of invasive catheterization techniques. A novel minimally invasive alternative can be based on contrast echocardiography by means of a trans-esophageal ultrasound (US) probe. Low concentrations of ultrasound contrast agent (UCA) are used in combination with low mechanical index (MI) US. For a correct application of this method, the relationship between UCA concentration and US intensity must be determined. To this end, a new calibration method developed by Yamada et al. was employed. The calibration measurements were used to determine the range of UCA concentrations which is the most suitable for accurate quantification.

Acoustic quantification of UCA is pursued in this work by employing specific contrast enhancement imaging modes. These methods allow improving the signal-to-noise ratio (SNR) by detecting the signal generated by UCA non-linear dynamics. During the calibration measurements it appeared however evident that the nonlinear propagation of US through UCAs may affect the image quality and reduce the SNR or, more specifically, the contrast-to-tissue ratio (CTR). Therefore, for an accurate acoustic quantification these non-linear effects have been measured and characterized together with their implications on contrast imaging.

A specific measurement setup was built for measuring nonlinear US distortion and its implications on contrast imaging. An US signal was sent using a single element transducer and detected by a calibrated hydrophone after passing through a latex tube filled with different concentrations of Luminity UCA (Bristol Myers Squibb, New York, USA). Different pulse sequences have been used to measure the effects on different contrast imaging US pulse sequences, namely power modulation (PM), second-harmonic, ultra-harmonic, and super-harmonic. Moreover, the nonlinear distortion was characterized by the Burger’s equation. Two different models to fit the measured US waves were therefore derived and used to estimated parameters to quantify the nonlinear distortion. All measurements were performed using a low MI of 0.1 and Luminity concentrations in the range of 0 to 18 μL/L. Three frequencies, 1, 1.5 and 2 MHz, which are near the resonance frequency of Luminity, were used.

A different setup was then used to determine the influence of nonlinear US propagation through different Luminity concentrations on different contrast imaging modes present in a standard US scanner. To this end, a tissue mimicking phantom (Urethane rubber) was adopted. Measurements were performed by both an iE 33 and a Sonos 5500 US scanner (Philips Medical Systems, Best, The Netherlands).

The calibration results showed a linear relation between Luminity concentration and backscatter intensity with determination coefficients $r^2 \geq 0.92$. The range of Luminity concentrations that resulted useful for contrast echography was comparable with earlier results and is in the range of 0 to 10.5 μL/L.

Nonlinear distortion measurements showed an increasing shift of spectral energy to the second harmonic frequency for increasing Luminity concentrations. The increase for other frequencies (sub- ultra- and super-harmonic) was less significant. For power modulation the increase of power for increasing Luminity concentration is most significant for sub- and ultra-harmonic frequencies. The results at 2 MHz where discarded due to the distortions introduced by the transducer.

The fit of the model showed a mean determination coefficient $r^2=0.99$ and the estimated attenuation and nonlinearity increased linearly with increasing UCA concentration for both frequencies. Regarding the imaging implications, a reduction of the CTR up to 60% was measured.
In general, the results prove the usability of contrast imaging for low MI echocardiography and low Luminity concentrations. However, especially for increasing concentrations, the effects of nonlinear US propagation through UCA on the image quality and the CTR should be considered. Further research could also focus on the integration of UCA bubble dynamics in the used model.
Samenvatting

Het kwantificeren van hart functioneren, vindt tegenwoordig nog steeds plaats middels invasieve katheterisatie technieken. Een nieuw, minimaal invasief alternatief kan worden gebaseerd op contrast echografie, met behulp van een trans-esophageal ultrasound (US) probe. Lage concentraties ultrasound contrast (UCA) worden gebruikt samen met een lage mechanische index (MI) US. Voor een correcte toepassing van de techniek is het noodzakelijk de relatie tussen contrast concentratie en US intensiteit te bepalen. Hiervoor werd een nieuwe kalibratie techniek gebruikt ontwikkeld door Yamada et al. Middels de kalibratie metingen werd het bereik van contrast concentraties bepaald geschikt voor akoestische kwantificatie.

De akoestische kwantificatie van UCA vindt in dit vakgebied plaats middels speciale contrast respons versterkende technieken. Deze technieken maken het mogelijk de signaal ruis verhouding (SNR) te verbeteren door het detecteren van de respons veroorzaakt door de niet lineaire UCA dynamica. Gedurende de kalibratie metingen werd het duidelijk dat de niet lineaire interactie tussen UCA en US van invloed zou kunnen zijn op de kwaliteit van de contrast respons versterkende technieken en de SNR kan verlagen, nog preciezer, de contrast-to-tissue ratio (CTR). Daarom is het voor een nauwkeurige akoestische kwantificatie van belang de niet lineaire effecten te meten en te karakteriseren, samen met de consequenties voor de contrast respons versterkende technieken.

Om de niet lineaire vervorming van US te meten, samen met de consequenties voor de contrast respons versterkende technieken werd een specifieke meetopstelling gebouwd. Een ultrasound puls werd verzonden met een single element transducer en gedetecteerd met een gekalibreerde hydrofoon na het passeren van een latex tube met Luminity oplossing (Brystol Myers Squibb, New York, USA). Verschillende puls sequenties zijn gebruikt om de consequenties voor verschillende contrast detectie puls sequenties te bepalen, namelijk power modulatie (PM), tweedeharmonisch, ultraharmonisch en superharmonisch. Verder werd de niet lineaire verstoring gekarakteriseerd door de Burger’s vergelijking. Hiervoor werden twee verschillende modellen afgeleid om de gemeten US golven te fitten en de parameters nodig voor de kwantificatie van de niet lineaire verstoring te schatten. Alle metingen werden uitgevoerd met een lage MI van 0.1 en Luminity concentraties in het bereik van 0 tot 18 μL/L. Drie frequenties, 1, 1.5 en 2 MHz welke dicht bij de resonantie frequentie van Luminity liggen, werden gebruikt.

Een andere meetopstelling werd gebruikt om de consequenties van de niet lineaire vervorming van US door verschillende Luminity concentraties te bepalen voor verschillende contrast detectie technieken beschikbaar op een gewone ultrasound scanner. Hiervoor werd een tissue mimicking fantoom (Urethane rubber) gebruikt. De metingen werden uitgevoerd met een iE33 and Sonos 5500 ultrasound scanner (Philips Medical Systems, Best, Nederland).

De kalibratie resultaten lieten een lineaire relatie tussen de Luminity concentratie en backscatter intensiteit zien, met een determinatie coëfficiënt $r^2 \geq 0.92$. Het gevonden bereik van Luminity concentraties bruikbaar voor contrast echography was vergelijkbaar met eerdere resultaten en is in het bereik van 0 tot 10.5 μL/L.

Uit de metingen van de niet lineaire verstoring van ultrasound bleek een toenemende verschuiving van spectrale energie van de tweedeharmonische frequentie voor oplopende Luminity concentraties. De toename voor ander frequenties (sub-, ultra, en superharmonisch) was minder significant. De resultaten van de metingen met 2 MHz kunnen niet worden gebruikt door een verstoring van het signaal veroorzaakt door de transducer.
Voor power modulatie is de toename van spectrale energie voor oplopende Luminity concentraties het meest significant voor sub- en ultraharmonische frequenties. De fit van het model laat een determinatie coëfficiënt zien van $r^2=0.99$. Voor beide frequenties neemt de verzwakking en niet lineairiteit lineair toe voor oplopende Luminity concentraties. Ten aanzien van de consequenties voor contrast detectie werd een reductie van de CTR tot 60% waargenomen.

In het algemeen bewijzen de resultaten de bruikbaarheid van contrast detectie technieken voor lage MI echocardiografie en lage Luminity concentraties. Maar, zeker voor oplopende concentraties, moet er rekening gehouden worden met de consequenties van de niet lineaire vervorming van de ultrasound op de image kwaliteit en de CTR. Verder onderzoek zou kunnen focussen op de integratie van UCA bubbel dynamica in het model.
# Content

1 Introduction ............................................................................................................. 11

2 Contrast echocardiography .................................................................................. 13
   2.1 Echography ........................................................................................................ 13
   2.2 US contrast agents ........................................................................................... 14
   2.3 Cardiac quantification ....................................................................................... 15

3 Calibration ............................................................................................................. 19
   3.1 Calibration method ............................................................................................ 19
   3.2 Calibration results ............................................................................................. 21
   3.3 Conclusions ........................................................................................................ 22

4 Nonlinear distortion of US ..................................................................................... 24
   4.1 Propagation through a nonlinear medium ......................................................... 24
      4.1.1 Nonlinear distortion .................................................................................... 24
      4.1.2 Attenuation ............................................................................................... 28
      4.1.3 Dispersion .................................................................................................. 30
   4.2 Propagation through a UCA .............................................................................. 30
      4.2.1 Bubble resonance ...................................................................................... 30
      4.2.2 Rectified diffusion .................................................................................... 32
      4.2.3 Rayleigh Plesset ...................................................................................... 32
      4.2.4 Extinction cross-section .......................................................................... 33
   4.3 Nonlinear propagation models .......................................................................... 34
      4.3.1 Nonlinear medium ..................................................................................... 34
      4.3.2 Bubble dynamics model ............................................................................ 39
   4.4 Nonlinear distortion measurements .................................................................... 41
      4.4.1 Measurement set-up ................................................................................... 41
      4.4.2 Analysis of the nonlinear distortion measurements .................................... 43
      4.4.3 Results of the nonlinear distortion measurements .................................... 44
      4.4.4 Conclusions ............................................................................................... 46

5 Imaging implications ............................................................................................... 48
   5.1 Contrast echography ......................................................................................... 48
      5.1.1 Harmonic Imaging ..................................................................................... 48
      5.1.2 Power and phase modulation ...................................................................... 49
   5.2 Backscatter by tissue of UCA generate nonlinearity ......................................... 50
   5.3 Imaging measurements ...................................................................................... 50
      5.3.1 Contrast imaging set-up .............................................................................. 50
      5.3.2 Imaging implications ................................................................................... 51
      5.3.3 Backscatter measurement set-up ................................................................ 52
1 Introduction

Measuring and interpreting bio-signals has always been the basis for medical diagnoses and determining a treatment. In cardiology there are several important cardiac parameters which still remain a challenge to measure accurately. Examples are the cardiac output (CO), ejection fraction (EF) and pulmonary blood volume (PBV) [21].

For a good interpretation of bio-signals it is important to measure them accurately, especially in a critical environment like an intensive care unit or operating room. In these critical environments cardiac quantification is done using invasive techniques based on catheterisation [21]. These are still the standard for cardiac quantification, because the available less invasive alternatives [21] have the drawback of a limited accuracy, being time consuming or in case of magnetic resonance imaging (MRI) [8] the large size of the scanner and the high magnetic field.

The practical limitations of cardiac quantification techniques using catheterization, because of their invasiveness, are the drive for the search for less invasive alternatives, which can be used in a critical environment.

One minimal invasive alternative for the simultaneous measurement of CO, EF and PBV is developed by Mischi et al. [21, 22]. This technique is based on the detection of the passage of a bolus of ultrasound contrast agent (UCA) at several sites in the central circulation using an ultrasound (US) transducer. Indicator dilution principles are used to interpret the measurements. Further development is done using a new UCA named Luminity (Bristol Myers Squibb, UK). In the USA Luminity is addressed as Definity. The theory behind contrast echography is given in chapter 2.

Signal processing is an important part of the measurement technique. It is used to improve the contrast to tissue ratio (CTR), which is a measure for the performance of the contrast detection. Signal processing is further used to interpret the noisy indicator dilution curve (IDC) measurements using specific models [21].

The use of indicator dilution principles requires knowledge of the course of nonlinear US distortion for increasing UCA concentrations, to show the consequences for contrast imaging. The contrast detection theory is given in chapter 5.

Before the consequences for contrast detection can be discussed, the nonlinear US distortion is determined for different Luminity concentrations. The measurements are performed using a calibrated single element transducer and several waveforms. A detailed description of the theory behind the nonlinear distortion of US, the measurement set-up and the results is given in chapter 0. The consequences for contrast imaging are discussed in chapter 5.

Next to the consequences of nonlinear US distortion on contrast detection, the consequences of the backscatter of nonlinear distorted US (due to UCA) by tissue are determined. The backscatter of nonlinear distorted US is measured for several contrast detection techniques and increasing Luminity concentrations. A trans-oesophageal (TEE) Omniplane III (Philips Medical Systems) US probe together with an urethane rubber tissue mimicking phantom (ATS Laboratories) are used to measure the backscatter of nonlinear distorted US by tissue. A description of the measurements setup and the results is given chapter 5.

Prior the nonlinear distortion measurements, the used range of UCA concentrations is determined using calibration measurements. These calibrations define the relation between UCA concentration and measured acoustic backscatter. A TEE Omniplane III (Philips Medical Systems) US transducer is employed using a calibration technique developed by Yamada et al [34]. A full description of the calibration method and the results is given in chapter 3.
2 Contrast echocardiography

The interest for the change of US nonlinearity for increasing UCA concentrations is due to a minimal invasive cardiac quantification method under development [21], based on contrast echocardiography. The strong potential of this method is the minimal invasive measurement of several cardiac parameters, like CO, EF and PBV, with one measurement.

This chapter gives the background on contrast echocardiography needed for the understanding of the nonlinear distortion of US and its influence on contrast detection. Paragraph 2.1 shows the basic principle of echography. A description of UCAs is given in paragraph 2.2. The contrast detection techniques are described in chapter 5. This chapter ends with the theory behind the cardiac quantification using contrast echography.

2.1 Echography

Before contrast echography can be discussed a short introduction in echography must be given. Echography uses acoustic waves (US) whose backscatter (echoes) are analyzed to obtain information about the tissue of interest. The acoustic waves are usually represented by the real part of the wave equation solution [12]

\[ RE[a] = a_0 \cdot \cos(k \cdot (v \cdot t - x)) \]  

Here \( a \) represents the amplitude of the wave, \( a_0 \) the maximum amplitude, \( k \) the wave number, \( v \) the propagation velocity and \( x \) the distance along the propagation axis.

\[ a_r (Z_2' \cos \beta_i - Z_1 \cos \beta_r) \]
\[ a_i (Z_2' \cos \beta_i + Z_1 \cos \beta_r) \]

\[ a_t = \frac{2Z_2 \cdot \cos \beta_i}{Z_2 \cdot \cos \beta_i + Z_1 \cdot \cos \beta_r} \]

Figure 2.1: Principle of echography, reflection of acoustic intensity due to a discontinuity in the acoustic impedance.
The angles \( \beta_i \) and \( \beta_r \), respectively represent the angle of incidence and the angle of reflection, \( Z_i \) represents the acoustic impedance of the medium, which is equal to [19]

\[ \sqrt{\rho \cdot c}. \quad \text{Eq. 2.4} \]

Here \( \rho \) is the density of the medium and \( c \) the US velocity in the medium.

The reflection of the US can also be expressed in terms of intensity instead of amplitude. The intensity hereby equals [21]

\[ I = \sqrt{\frac{I_i}{Z \cdot (2 \cdot \pi \cdot f \cdot a)^2}}, \quad \text{Eq. 2.5} \]

with \( f \) the US frequency.

Combining the equations 2.2, 2.3 and 2.5 gives the reflected and transmitted intensities as

\[ \frac{I_r}{I_i} = \left( \frac{a_r}{a_i} \right)^2 = \left( \frac{Z_2 \cdot \cos \beta_i - Z_1 \cdot \cos \beta_r}{Z_2 \cdot \cos \beta_i + Z_1 \cdot \cos \beta_r} \right)^2, \quad \text{Eq. 2.6} \]

\[ \frac{I_t}{I_i} = \frac{Z_2}{Z_1} \left( \frac{a_r}{a_i} \right)^2 = \frac{4 \cdot Z_1 \cdot \cos \beta_i \cdot Z_2 \cdot \cos \beta_i}{(Z_2 \cdot \cos \beta_i + Z_1 \cdot \cos \beta_r)^2}. \quad \text{Eq. 2.7} \]

Equations 2.6 and 2.7 are based on the energy conservation principle, which the US intensity has to satisfy, meaning that the reflected and transmitted intensities together equal the intensity of the incidence US.

The conversion of the acoustic energy into electrical energy and vice versa is done by a piezoelectric crystal in the transducer. This conversion is approximately linear [21].

The mechanical interaction of the US, in this case with the contrast bubbles, is expressed by the MI. The MI depends on the peak negative US pressure \( (P_{neg}) \) and the US frequency and is calculated as [19]

\[ MI = P_{neg} \cdot f^{-\frac{1}{2}}. \quad \text{Eq. 2.8} \]

An US scanner sends US pulses to analyze the US echoes (backscatter). The delay between the echoes then can be interpreted in terms of distance. Due to the relative small differences in acoustic impedance between most tissues in the human body (except for bone tissue and lungs) US can penetrate relatively deep into the body and give information about deeper lying tissue.

To address the difference between US propagating through the tissue and the echoes (backscatter) measured at the US transducer, the US intensity measured at the probe is referred to as US backscatter.

### 2.2 US contrast agents

The modern UCA used nowadays are based on small bubbles with a diameter between 1 \( \mu \)m and 10 \( \mu \)m of an inert gas (e.g., per-fluorocarbons) encapsulated in a bio-compatible shell (e.g., phospholipids). The shell and the low solubility of per-fluorocarbons protect the bubbles from dissolving into the blood. The small sizes of the bubbles, most bubbles smaller then a red blood cell (6 / 8 \( \mu \)m), lets them pass through the lung capillaries.
The interaction of a UCA bubble with US differs dependent of the MI. A more detailed description on the MI dependence of the bubble's response is given in paragraph 4.2.

The nonlinear backscatter due to a contrast bubble is defined by the nonlinearity coefficient $\beta$, which is the ratio between the nonlinear US scattered in all directions and the incident fundamental acoustic intensity. The US backscatter is the strongest when the frequency is the resonance frequency ($f_r$), which has an inverse relation with the UCA bubble radius ($R_0$). The attenuation of the US is defined by the attenuation coefficient ($\alpha$). The attenuation coefficient represents the loss of acoustic pressure along the distance that the US beam covers through the contrast dilution. A more detailed description of $\alpha$ and $\beta$ is given in paragraph 4.2.

For high contrast concentrations the attenuation causes shadowing, meaning that eventually all US energy is blocked by the first part of the UCA hiding the deeper part. An example of shadowing is shown in figure 2.2.

![Figure 2.2: Shadowing effect shown with fundamental imaging. Left without UCA. Right with Luminity UCA (67.5 μL/L).](image)

Several properties of the Luminity [3] UCA are shown in table 2.1.

<table>
<thead>
<tr>
<th>Luminity</th>
<th>Shell</th>
<th>Gas</th>
<th>Size distribution</th>
<th>Activation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liposome</td>
<td>$C_3F_6$, 150 μL/mL</td>
<td>Mean 1.1-3.3 μm 98% &lt; 10 μm Max 20 μm</td>
<td>Using a Vail mix to shake the Luminity vial for 45 s.</td>
</tr>
</tbody>
</table>

### 2.3 Cardiac quantification

The, by Mischi et al. [21, 22] developed, cardiac quantification technique is based on a rapid indicator dilution technique. A small bolus of UCA is injected in a peripheral vein and the passage of the bolus is detection at several sites in the central circulation. Rapid indicator dilution techniques use a rapid injection of an indicator bolus to produce a short IDC measurement. The use of a bolus of indicator leads to a small dose administered to the patient, which leads to less strict requirements for the indicator compared to continues indicator dilution techniques.

The principle of rapid indicator dilution techniques is based on the measurement of the dilution of the indicator when travelling through the body. The indicator dilution principle is illustrated using the determination of the mean flow $\phi$. The determination
of other parameters is explained by Mischi et al. [21]. The concentration of the indicator is measured after the injection point, as shown in figure 2.3.

![Indicator bolus injection](image)

**Figure 2.3**: Scheme of a rapid injection method together with the indicator concentration versus time (IDC). The second rise is due to recirculation.

Due to the injection of an indicator bolus, the concentration is not constant in time. The measurement of the spread of indicator concentration over time ($O(t)$) is referred to as the IDC.

Rapid injection techniques use several assumptions to determine the flow. The first is a constant flow during the time of the measurement, which is approximately one minute. Furthermore it is assumed that there is instantaneous and uniform mixing of the indicator in the blood, the injection is so fast that it can be modelled by a Dirac pulse and the loss of indicator is absent or known.

The injected indicator dose is now determined by the following integral [21]:

$$m_i = \int_0^\infty O(t) dt \Rightarrow \phi = \frac{m_i}{\int_0^\infty O(t) dt},$$

Eq. 2.9

which is referred to as the Stewart-Hamilton equation.

There are two phenomena that complicate the IDC analyses. The first is recirculation of the indicator. Due to the recirculation, part of the tale of the first pass of the indicator is covered by the recirculation. An example of the effect of recirculation on the IDC is show in figure 2.3. The second is the often relative high noise present during the concentration measurements, resulting in a noisy IDC. These two phenomena can be reduced by using a model to analyze the IDC.

Two types of US probes are available for the detection of the UCA bolus in the central circulation. A trans-thoracic (TTE) US probe, which records the US backscatter from the outside of the body, and a TEE US probe, which is records the US backscatter from the oesophagus. TEE imaging has the advantage of much less tissue between the US probe and the central circulation, which reduces the patient dependency of the measurements and increases the reproducibility between patients. A disadvantage of TEE imaging is the discomfort for a non sedated patient, because the TEE US probe has to be inserted in the oesophagus.
After calibration of the relation between UCA concentration and acoustic backscatter intensity, the acoustic intensity detected by an US scanner allows the measurement of an UCA dilution curve. The measured dilution curve is modelled and interpolated, to reduce the effects of noise and recirculation of the UCA, before cardiac quantification is performed.

The dilution curve modelling is done using models which give a physical characterization of the dilution process, like a local density random walk (LDRW) [21] or first passage time model (FPT) [21]. The LDRW model is preferred, because it showed slightly better fits and allows distinction between the mean residence time (MRT) and the mean transit time (MTT) as explained by Mischi et al. [21, 22].

The fitting of the model on the measurements is done using multiple linear regression along the dilution curve segment showing no recirculation as explained by Mischi et al. [21].
3 Calibration

The calibration measurements are performed for three reasons. The first, and most important, reason is determining the relation between Luminity concentration and acoustic backscatter. This relation is essential to determine the IDC from the measured acoustic intensity, and thus essential for cardiac quantification using indicator dilution principles described in paragraph 2.3. The acoustic backscatter measurement also shows the Luminity concentration where shadowing gets noticeable, from this the concentration range which can be used for cardiac quantification can be determined. The second reason is to compare the performance of the new calibration method with previous calibration results. The third reason for the calibrations is to compare the performance of two different US scanners using low concentrations of UCA and a low MI.

The chapter starts with a description of the calibration method and uncertainty factors in paragraph 3.1. Paragraph 3.2 gives the results of the different calibration measurements. The chapter ends with the conclusions from the calibration results.

3.1 Calibration method

The most important reason for the calibration measurements, determining the relation between Luminity concentration and acoustic backscatter, requires the measurement of the acoustic backscatter for a range of Luminity concentrations.

The concentration range should be between zero and the first concentrations where shadowing becomes noticeable to determine the concentration range for an undisturbed derivation of the IDC. Previous calibrations with Luminity [15, 34] showed noticeable shadowing for concentrations above approximately 0.001 times the concentration in the vial, implying that only very low concentrations of Luminity can be used for cardiac quantification using IDC measurements.

The used calibration set-up is an adapted version of the one described by Yamada et al. [34]. A new calibration set-up was introduced because of large variations found during calibrations using the previous set-up [15].

For the acoustic backscatter measurement an Omniplane III (Philips Medical systems) TEE probe is used. The TEE approach promises a decreased patient dependency of the calibration and, therefore, improves the usability of the measurement technique as mentioned in paragraph 2.3.

To minimize US reflections from the walls of the beaker glass, the inside is covered with acoustic absorbing material as shown in figure 3.1.

![Diagram](image)

**Figure 3.1 : Scheme of the calibration set-up.**
The different Luminity dilutions are made following the calibration protocol shown in appendix A.

The concentrations of the dilutions are expressed in μL/L and represent the amount of C₃F₆ gas in μL per litre saline. The concentrations used for the calibration are listed in appendix A and are made using a physiological saline dilution (0.9% NaCl) minimizing the amount of air bubbles to reduce measurement disturbances.

The used volumes of saline have to be measured precisely, because any volume error causes a concentration error of the same order. Due to the small concentrations of interest, up to three dilution steps are necessary to reach the smallest concentrations. In these three dilution steps the concentration errors add up.

During the measurement the dilutions are stirred using a magnetic stirrer to ensure a uniform mixing of the dilutions. Figure 3.2 shows photos of the set-up and the dilutions.

![Figure 3.2: Photos of the set-up and the dilutions.](image)

To minimize the destruction of Luminity bubbles, all measurements are performed with a low MI of 0.1 and the exposure to the US is kept short in time.

The calibrations are performed using two different US scanners and two different imaging modes, TCE harmonic and TCE ultra-harmonic imaging using a Sonos 5500 (Philips Medical systems) and tissue harmonic imaging using an iE33 (Philips Medical Systems). These modes are chosen to compare the calibration results for the two US scanners (harmonic imaging) and to compare the results of the new calibration method with the best results from previous calibrations [15] (ultra-harmonic imaging). No other imaging modes are used, because it is not known if they are comparable between the two US scanners.

The setting of the US machine and the measurement set-up are kept constant for all calibrations. The used settings, shown in the calibration protocol in appendix A, are chosen because they provide a clear image on the US scanner.

The quantification of the measured acoustic backscatter in the region of interest (ROI) is performed using Qlab (Philips Medical Systems). The relation between the Luminity concentration and the acoustic backscatter is analysed using Matlab (the Mathworks). The data fitting is done using the LSE fitting algorithm available in Matlab. The correlation between the fit and the measured acoustic intensity is expressed with the

![Figure 3.3: ROI Placement.](image)
The determination coefficient $r^2$.

The ROI for the measurement of the acoustic backscatter is placed immediately beyond the near-field zone as shown in figure 3.3. This is done to avoid near-field artefacts and to minimize the effects of shadowing.

Earlier calibrations with SonoVue [21] and Luminity [15, 34] showed a linear relation between the Luminity concentration and acoustic backscatter for concentrations with no noticeable shadowing. Due to these results the expectation for the calibration measurements is a relation between Luminity concentration and acoustic backscatter with a similar linearity. The SonoVue results can be used for this expectation, because both SonoVue and Luminity are third generation contrast agents with a similar structure (Phospholipid shell).

The performance of the different imaging modes and US scanners are compared using the determination coefficient of the linearity and the linear range.

### 3.2 Calibration results

After analysis with Matlab, the calibration results with Luminity show a linear relation between acoustic intensity and UCA concentration with a determination coefficient between 0.92 and 1.00 for the different imaging modes and US scanners. This linear part covers concentrations from 0 up to 9.6 $\mu$L/L for harmonic imaging and 0 up to 4.8 $\mu$L/L for ultra-harmonic imaging.

For all harmonic calibrations, shadowing starts to become noticeable for concentrations equal or above 19.5 $\mu$L/L. For ultra-harmonic imaging shadowing starts to become noticeable for concentrations equal or above 9.6 $\mu$L/L.

Table 3.1 and figure 3.4 show an overview of the Luminity calibration results.

![Figure 3.4: Overview of the Luminity calibration results.](image-url)
Table 3.1: Overview of the Luminity calibration results.

<table>
<thead>
<tr>
<th>Imaging Mode</th>
<th>Scanner</th>
<th>Linear Range</th>
<th>Linearity</th>
<th>Shadowing</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCE Harmonic</td>
<td>Sonos 5500</td>
<td>0 µL/L - 4.8 µL/L</td>
<td>$r^2 = 0.95$</td>
<td>$\geq 19.5 \mu$L/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 µL/L - 9.6 µL/L</td>
<td>$r^2 = 0.92$</td>
<td>$\geq 19.5 \mu$L/L</td>
</tr>
<tr>
<td>Tissue Harmonic</td>
<td>iE33</td>
<td>0 µL/L - 9.6 µL/L</td>
<td>$r^2 = 0.97$</td>
<td>$\geq 19.5 \mu$L/L</td>
</tr>
<tr>
<td>TCE Ultra-Harmonic</td>
<td>Sonos5500</td>
<td>0 µL/L - 4.8 µL/L</td>
<td>$r^2 = 1.00$</td>
<td>$\geq 9.6 \mu$L/L</td>
</tr>
</tbody>
</table>

3.3 Conclusions

As expected from previous calibrations [15, 21, 34] all calibrations show a linear relation between Luminity concentration and acoustic backscatter for concentrations from zero till shadowing becomes noticeable.

The calibrations using harmonic imaging show a linear relation with a determination coefficient between 0.92 and 0.97. These high determination coefficients show a good linear relation between acoustic backscatter and Luminity concentration, proving the suitability of harmonic imaging for IDC measurements. All harmonic calibrations show an initial shadowing at concentrations equal or above 19.5 µL/L, which is comparable with the results found by Yamada et al. [34] and previous calibrations [15].

The best results with harmonic imaging were found using the iE33 US scanner, because these show the highest determination coefficient and the largest linear range, as shown in table 3.1.

The calibrations using ultra-harmonic imaging show a linear relation with a determination coefficient of 1.00, but only for a concentration range between 0 and 4.8 µL/L. This is a better determination coefficient than found using harmonic imaging, but for a smaller concentration range. Despite the smaller range, ultra-harmonic imaging can still be reported as suitable for IDC measurements.

Comparing the ultra-harmonic calibration results with previous calibrations using ultra-harmonic imaging [15], which showed a linear relation with a mean determination coefficient of 0.94, shows a better linear relation using the new calibration method. Due to the large variations in found determination coefficients and linear ranges during the previous calibrations [15], it is not possible to conclude that the new calibration method performs better. More calibrations are needed to conclude which calibration method shows the best performance.

Comparing the calibration results, shown in table 3.1, with the results from Yamada et al. [34] it can be concluded that our calibrations show a linear range which is comparable, but with a smaller determination coefficient. Only for the smaller range between 0 and 4.8 µL/L ultra-harmonic imaging performs equally well.

Since only one set of calibrations is performed, it is not possible to look at the improvement of the reproducibility using the new calibration method. Further calibrations are necessary and planned already to investigate reproducibility and to enforce the found results.


4 Nonlinear distortion of US

The influence of tissue or water on propagating US is often described using linear models. Linear models are used, because of their low complexity and an accuracy, which is sufficient for most applications. However, the propagation of US through tissue or water remains a nonlinear process.

In the context of contrast echocardiography, the nonlinearity of tissues and water is much smaller than the nonlinearity introduced by the UCA as shown in paragraph 4.1. Although UCAs introduce the dominant nonlinearity for contrast echocardiography, this chapter starts with a description of nonlinear propagation in tissue or water in paragraph 4.1. This is done, because these nonlinearities help explain the nonlinear US distortion due to UCAs, discussed in paragraph 4.2. The propagation of US through an UCA dilution is a complex process due to the interactions between different nonlinear properties present along the path of the US through tissue and UCA.

The strong nonlinearity of UCA results in the need for nonlinear models to describe the US distortion. Paragraph 4.3 discusses several nonlinear models describing the different nonlinearities and their interactions. The aim of the nonlinear propagation models is to give a simple, but accurate, representation of the nonlinear US distortion.

The experimental set-up and results to determine the nonlinear US distortion, for low UCA concentrations and low MI, are given in paragraph 4.4. These experiments also provide information about the accuracy of the different models.

4.1 Propagation through a nonlinear medium

In contrast echocardiography the US waves from the transducer pass through several types of tissue and the UCA dilution before the backscatter reaches the transducer. The nonlinear properties of the different tissue types are cumulative and build up as the US passes through the tissue.

The nonlinearity of tissue includes several interacting properties, which are described in sections 4.1.1 till 4.1.3 together with their interactions.

4.1.1 Nonlinear distortion

The nonlinear distortion of propagating US depends on the nonlinear relation between the density variation of the medium (p') and the acoustic pressure variation (P'). Due to this nonlinear relation the energy from the US waveform, present at the fundamental (send) frequency, is spread over a range of frequencies round the fundamental frequency. This is a cumulative process, spreading more energy over a wider frequency range for every small distance step the US passes through the tissue.

The nonlinear relation between p' and P' is expressed in the equation of state

\[ P = P(p, S) \]

with S being the entropy. A Taylor expansion\(^1\) describing the equation of state without attenuation is defined as [2, 11, 23, 25]

\(^1\)The acoustic pressure in a medium is a function of the density and entropy \( P(p, S) \). The Taylor expansion is made around \( S = S_0 \). The partial derivatives from equations 4.1 are evaluated at the initial state \( (p_0, S_0) \).
With \( P' = P - P_0, \) \( \rho' = \rho - \rho_0 \) and the subscript 0 denoting the unperturbed value of \( \rho \) and \( P. \)

In literature \([2, 20, 23, 25]\) it is shown that for a plane progressive wave, which is a feasible assumption for low MI and low UCA concentration contrast echocardiography, a Taylor expansion of the second order gives an accurate description of the equation of state and is solvable. The experimental results, shown in section 4.4.3 enforce this assumption. Limiting equation 4.1 to the second order and combining it with the definition of the linear equation of state \([2]\), which is defined as

\[
P' = c_0^2 \cdot \rho',
\]

results in the following approximated equation of state:

\[
P' = c_0^2 \cdot \rho' + \frac{1}{2!} \left( \frac{\partial^2 P}{\partial \rho^2} \right)_S \cdot \rho'^2.
\]

Here \( c_0 \) is the unperturbed US speed which in water has a value of approximately 1500 m/s.

To derive a measure for the nonlinearity introduced by the tissue it is necessary to write the equation of state from equation 4.3 as a function of amplitude:

\[
P' = A \cdot \left( \frac{\rho'}{\rho_0} \right) + \frac{B}{2!} \cdot \left( \frac{\rho'}{\rho_0} \right)^2.
\]

With \( A \) and \( B \) representing the amplitudes of the fundamental and second harmonic components respectively. The ratio \( \frac{B}{A} \) can now be used as a simple measure for the amount of nonlinearity introduced by the tissue. For the measurement of \( \frac{B}{A} \) it is necessary to vary \( \rho' \) adiabatically to measure the change in \( P' \). This is a difficult task for liquids and tissues, because of their low compressibility \([2]\). More commonly used is the coefficient of nonlinearity \( \beta \) defined as

\[
\beta = 1 + B \cdot (2A)^{-1}.
\]

Later in this paragraph it will be explained why the nonlinear parameter \( \beta \) is more easy to use.

Comparing Equations 4.3 and 4.4 term to term gives the following physical representation for the variables \( A \) and \( B \):

\[
A \cdot \left( \frac{\rho'}{\rho_0} \right) = c_0^2 \cdot \rho' \rightarrow A = c_0^2 \cdot \rho_0
\]

\[
\frac{B}{2!} \cdot \left( \frac{\rho'}{\rho_0} \right)^2 = \frac{1}{2} \left( \frac{\partial^2 P}{\partial \rho^2} \right)_S \cdot \rho'^2 \rightarrow B = \rho_0^2 \cdot \left( \frac{\partial^2 P}{\partial \rho^2} \right)_S.
\]
Combining the definitions for $A$ and $B$ with Equation 4.1 gives the following physical representation of the equation of state

$$ P' = c_0^2 \cdot \rho' + \left( B \cdot \left( 2A \right)^{-1} \cdot c_0^2 \cdot \rho_0^{-1} \right) \cdot \rho'^2. \tag{4.7} $$

Most soft types of tissue, just like water, have a $\beta$ between 3.5 and 6.5 as shown in figure 4.1. The $\beta$ of a UCA can be more than thousand times larger than for tissue, depending on the properties of the UCA. The nonlinearities introduced by UCA are described in paragraph 4.2.

![Figure 4.1: Coefficients of nonlinearity for water and different tissue types.](image)

The nonlinear deformation of a single frequency, sine wave US signal due to cumulative nonlinear distortion can be split into different stages as shown figure 4.2. The first stage is the single frequency sine wave emitted by the US transducer. Here no deformation is present. When the US passes through the tissue the second stage is reached and nonlinear distortion is introduced.

Next derivation of the influence of the nonlinear relation between $\rho'$ and $P'$ on the US speed ($c$) will show that the nonlinearity parameter can be determined using the change in US speed. The relation between $c$, $\rho$ and $P$ is defined as [2]

$$ c^2 = \frac{\partial P}{\partial \rho}. \tag{4.8} $$

Combining the time derivative of equations 4.7 with the definition of $c^2$ from equation 4.8 results in

$$ c^2 = c_0^2 \cdot \left( 1 + B \cdot A^{-1} \cdot \rho' \cdot \rho_0^{-1} \right). \tag{4.9} $$

Taking the square root of equation 4.9 and performing a binomial expansion to show the influence of $\beta$ on $c$ gives

$$ c = c_0 \cdot \left( 1 + B \cdot \left( 2A \right)^{-1} \cdot \rho' \cdot \rho_0^{-1} \right). \tag{4.10} $$

Using the relation between density and particle speed variation ($v'$)

$$ \rho' \cdot \rho_0^{-1} = v' \cdot c_0^{-1}, \tag{4.11} $$

results in

$$ c = c_0 + B \cdot \left( 2A \right)^{-1} \cdot v'. \tag{4.12} $$
The influence of $\frac{B}{A}$ on the US speed can also be expressed as a function of $P'$ using equation 4.43. The reason why this linear relation can be used is explained later in this section. Equation 4.12 now becomes

$$c = c_0 + B \cdot (2A)^{-1} \cdot (\rho_0 \cdot c_0) \cdot P'.$$

Eq. 4.13

The dependence shown in equation 4.13 between the nonlinearity of the medium, the acoustic pressure and the US speed result in a difference between the speed in the compressional (positive) and rarefactional (negative) half cycle of the US wave. This speed difference between the two half cycles introduces a distortion of the sine wave as it propagates through nonlinear media. This phenomenon is easier to measure. From equation 4.13 it is clear that $\beta$ can be derived from the influence of the medium on the US speed, which is why $\beta$ is preferred above $\frac{B}{A}$.

![Diagram](image_url)

A sine wave pulse with freq. $f$ is transmitted by the transducer.

Due to the nonlinear relation between pressure and US speed shown in equation 4.13 the velocity in the compressional phase ($v_c$) is larger than that of the rarefational phase ($v_r$), the sine wave is distorted and harmonics are introduced.

The velocity difference creates a "saw tooth" wave, which is rich in harmonics. This eventually leads to acoustic saturation of the medium.

![Graph](image_url)

Figure 4.2: The different stages of nonlinear deformation due to a nonlinear medium.

The distortion of the sine wave broadens the frequency spectrum, spreading the power of the US over a broader spectrum when the US passes a larger distance through the medium. The harmonic frequencies (including ultra- and sub harmonic frequencies) that are formed due to the broadening of the spectrum are of special interest. The more distortion is introduced, the broader the frequency spectrum and
the more energy from the fundamental frequency \( f \) is spread over the broadened spectrum.

The distortion of the US wave is at its maximum when the wave has reached a saw tooth shape, as shown in the third stage of figure 4.2. When the wave travels further through the tissue, the nonlinear effects can no longer be described without the attenuation properties of the tissue. These will be described in the section 4.1.2.

As shown in equation 4.13 the nonlinear deformation becomes stronger for larger \( \beta \), larger MI of the US (larger \( P_0 \)) and when the US travels a longer distance through the tissue.

### 4.1.2 Attenuation

In the above description of nonlinear US distortion a lossless medium was assumed. This assumption in reality is never true and was only made to simplify the explanation. Attenuation reduces the energy present in the US wave when travelling through the medium and is caused by thermal and viscous losses.

The equation of state introduced in equation 4.7 can be expanded to include an attenuation term. The attenuation term describes the effect the entropy of the medium has on the pressure of the US. According to the literature [2, 11, 23, 25] it is of the form

\[
\left( \frac{\partial P}{\partial S} \right)_\rho \cdot S'
\]

Eq. 4.14

Adding this attenuation term to equation 4.7 gives

\[
P' = c_0^2 \cdot \rho' + \left( \frac{B}{2A} \cdot \frac{c_0^2}{\rho_0} \right) \cdot \rho'^2 + \left( \frac{\partial P}{\partial S} \right)_\rho \cdot S'.
\]

Eq. 4.15

To reduce the equation of state to a function of \( \rho' \) and \( P' \) the entropy \( (S') \) is expressed as a function of temperature \( (T') \) using a linear approximation of the heat transfer equation [2, 11, 23, 25]

\[
\frac{\partial S'}{\partial t} = K \cdot (\rho_0 \cdot T_0 \cdot c_0^2)^{-1} \cdot \frac{\partial^2 T'}{\partial t^2} \rightarrow S' = K \cdot (\rho_0 \cdot T_0 \cdot c_0^2)^{-1} \cdot \frac{\partial T'}{\partial t}.
\]

Eq. 4.16

Here \( K \) represents the heat conductivity number.

\( T' \) Can be expressed as a function of \( \rho' \) using the thermo dynamical relationship [2, 11, 23, 25]

\[
T' = T_0 \cdot \vartheta \cdot c_0^2 \cdot (\rho_0 \cdot C_p)^{-1} \cdot \rho',
\]

Eq. 4.17

with \( \vartheta \) representing the thermal expansion coefficient of the medium.

The thermo dynamical relationship [2, 11, 23, 25]

\[
\left( \frac{\partial P}{\partial S} \right)_\rho = \vartheta^{-1} \cdot (C_p \cdot C_v^{-1} - 1) \cdot \rho_0,
\]

Eq. 4.18

with \( C_p \) and \( C_v \) representing the specific heat at constant pressure and volume respectively, can be used together with the equations 4.16 and 4.17 to rewrite the equation of state as
$$p' = c_0^2 \rho' + \left( B \cdot (2A) \cdot c_0^2 \cdot \rho_0^{-1} \right) \rho'^2 - K \cdot \left( \rho_0 \cdot c_0^2 \right) \frac{\partial \rho'}{\partial t}. \quad \text{Eq. 4.19}$$

Due to the frequency dependency of $\frac{\partial \rho'}{\partial t}$, which is shown below, the attenuation acts as a low pass filter reducing the energy present at higher frequencies more than the fundamental (transmitted) frequency of the US wave. This frequency dependency of the attenuation reduces the deformation of the US due to nonlinearity of the medium.

A measure for the interaction between nonlinear distortion and absorption is the Gol’dberg number, which is defined as

$$\Gamma = \sigma \cdot (\alpha \cdot z)^{-1}. \quad \text{Eq. 4.20}$$

Here $z$ is the displacement of the US, $\alpha$ is the thermal and viscous attenuation coefficient of tissue defined as

$$\alpha = -\delta \cdot \omega^2 \cdot \left( 2 \cdot c_0^2 \right)^{-1}, \quad \text{Eq. 4.21}$$

with $\delta$ representing the diffusivity of the US and $\sigma$ is the normalized distance parameter, which is a measure for the introduced nonlinearity by the tissue and is defined as

$$\sigma = \beta \cdot \varepsilon \cdot k \cdot z = \omega \cdot \beta \cdot P_a \cdot z \cdot \left( \rho_0 \cdot c_0^3 \right)^{-1}. \quad \text{Eq. 4.22}$$

Here $\varepsilon$ is the acoustic Mach number defined as

$$\varepsilon = P_a \cdot \left( \rho_0 \cdot c_0^2 \right)^{-1}, \quad \text{Eq. 4.23}$$

with $\omega$ the angular frequency, and $k$ the wave number defined as

$$k = \omega \cdot c_0^{-1}. \quad \text{Eq. 4.24}$$

The definition of $\alpha$ shows a quadratic dependency on frequency, meaning that attenuation is a nonlinear process by itself.

For $\Gamma < 1$ the low pass filtering of the absorption prevents significant nonlinear distortions from developing. For $\Gamma > 1$ the nonlinear distortion becomes more dominant than the absorption.

When comparing the Gol’dberg number of water with those of tissue, as shown in figure 4.3, it can be seen that water has a much larger Gol’dberg number. Because the nonlinearity of tissue and water is in the same dimension, as shown in figure 4.1, this means that water has a much lower $\alpha$ than tissue. Thus US in water is much less attenuated than in tissue and the nonlinear deformation is much less affected by attenuation.
4.1.3 Dispersion

The last source of nonlinear distortion is dispersion. Which for water and tissue is often negligible. Dispersion is the dependence of the phase speed on frequency and it is related with attenuation [7]. Due to the relation between dispersion and attenuation, dispersive media also show frequency dependent attenuation.

In media where the dispersion is strong in comparison with the nonlinearity, large variations in phase speeds (caused by the dispersion) in combination with frequency depended attenuation (which is stronger for higher frequencies), limit the amount of energy transferred from the fundamental frequency of the US wave to other frequencies. This limits the nonlinear distortion of the US.

Bubbly liquids like UCA dilutions show strong dispersion, in most other media dispersion is negligible.

4.2 Propagation through a UCA

The use of an UCA during echocardiography adds the nonlinear properties of the UCA on top on the nonlinearities of the tissue (mostly blood), with the nonlinearities of the UCA being potentially thousand times stronger than those of tissue. The nonlinear properties of an UCA are described in the sections 4.2.1, 4.2.2, 4.2.3 and 4.2.4.

4.2.1 Bubble resonance

The large backscatter from an UCA dilution is not due to the difference between the acoustic impedance of blood and the gas inside the UCA bubbles, because the size of the UCA bubbles is much smaller than the US wavelength. The backscatter is caused by the radiation of US due to bubble oscillations.

For very low MI US (MI < 0.1, [1]), the UCA bubbles expand and contract with the rhythm of the compressional and rarefactional half cycle of the US wave as shown in figure 4.4. These small forced oscillations of the UCA bubbles introduce a linear backscatter.

The response of the bubble is controlled by the compressibility of the encapsulated gas, the properties of the shell and the inertia of the medium pushing on the surface of the bubble. The response of UCA bubbles is optimal for the resonance frequency \( f_r \), which is also dependent on the size distribution of the bubbles. The frequency \( f_r \) of a shell encapsulated bubble can be approximated by

\[
 f_r \approx \left(2 \cdot \pi \cdot R_0 \right)^{-1} \sqrt{3 \cdot \gamma \cdot \rho^{-1} \left(P_0 + 2 \cdot \sigma_{\text{eff}} \cdot R_0^{-1} \right)}
\]

Eq. 4.25
and is based on the resonance frequency of the free gas bubble \( f_{r,\text{free}} \) as shown by De Jong et al. [16]. The resonance frequency \( f_{r,\text{free}} \) of a free bubble can be approximated by

\[
f_{r,\text{free}} \approx 2 \cdot \pi \cdot R_0 \sqrt{3 \cdot \gamma \cdot P_0 \cdot \rho^{-1}}.
\]

Eq. 4.26

Here \( R_0 \) is the equilibrium radius of the bubble, \( \gamma = C_p / C_v \) is the ratio of heat capacities at constant pressure \( (C_p) \) and constant volume \( (C_v) \), and \( \sigma_{st} \) is the surface tension of the bubble. For Luminity, \( f_r \) is in the order of 1.5 MHz [27, 28].

When the MI of the US is increased to the range of middle MI (approximately \( 0.1 \leq \text{MI} \leq 1 \), [1]) the UCA bubble can expand with the US field, but its contraction is limited by the volume of encapsulated gas. This results in an asymmetric pressure response of the bubble as a function of time as shown in figure 4.4. The asymmetric pressure response is a nonlinear response and broadens the frequency spectrum.

The shell stabilizing the UCA bubble tempers the nonlinear response in comparison with that of a free air bubble, due to the extra dampening that it introduces. The dampening works in two ways: it increases the pressure needed to fully compress
the UCA bubble and it limits the expansion of the bubble, creating a more symmetric oscillation.

For an US field with a high MI (approximately MI > 1 [1]), transient or inertial cavitations occurs, which is the rapid growth and violent destruction of the UCA bubbles. Due to the low MI that is commonly used in perfusion imaging, the violent destruction of Luminity bubbles is minimal.

4.2.2 Rectified diffusion

The resonance property of an UCA depends on the size distribution of the bubbles. This size distribution changes in time due to the diffusion of gas from the UCA bubble into the surrounding blood, shrinking the bubble. This diffusion lets free gas bubbles dissolve in microseconds.

Shell encapsulated gas bubbles protect the gas from diffusing out of the bubble, slowing down the dissolving process of UCA bubbles.

Under influence of US [16, 19, 24] the diffusion process can be turned around, letting the bubble grow. This process is called rectified diffusion and influences the response of the bubble to US, due to the increasing bubble size.

During the compressional half cycle, the gas pressure inside the bubble is high and the surface area for the gas to enter the bubble is small due to the smaller size of the bubble. This smaller size also thickens the shell, further reducing the diffusion of gas. During the rarefractional half cycle, the gas pressure in the bubble drops and the surface area for the gas to enter the bubble is increased, thinning the shell. This creates a pressure gradient to the inside of the bubble. For free air bubbles the growth of bubbles is rapid, resulting in the violent collapse of the bubble. For shell encapsulated gas bubbles this process is much slower. The effect of rectified diffusion on Luminity during echocardiography is assumed negligible, due to the short sonification time (due to flow) and the low MI.

When the bubble radius is increased beyond a certain threshold the elasticity of the bubble shell gets compromised. Due to this the shell gets floppy when the amount of gas inside the bubble is reduced due to diffusion. The assumption of spherical bubble oscillations does not hold for these floppy bubbles. When using low MI US and short sonification times the number of floppy bubbles is assumed negligible.

4.2.3 Rayleigh Plesset

The response of an USA bubble due to US can be modelled using the modified Rayleigh Plesset equation [17].

The modified Rayleigh Plesset equation assumes spherical UCA bubbles, filled with an ideal gas and surrounded with an infinite medium. Although these assumptions are not met in reality, the errors caused are small. New research is being performed to incorporate non spherical bubbles in the Rayleigh Plesset equation. Furthermore it is assumed that responses of the UCA bubbles due to US do not influence each other, and that the US wavelength is much larger than the UCA bubble diameter, which is realistic for low concentrations of Luminity in combination with a low MI. Rectified diffusion is assumed to be absent, which is realistic due the short sonification during echocardiography.

The UCA bubble response due to US can therefore be modelled as [17]

\[ \rho \cdot R \cdot \dot{R} + \rho \cdot \frac{3}{2} \cdot R \ddot{R} = p_{\infty}(R_0 \cdot R^{-1})^2 + p_v - P_0 - 2 \cdot \sigma \cdot R^{-1}, \]

\[ -S_f(R_0^3 - R^3) - \delta_t \cdot \omega_0 \cdot \rho \cdot R \cdot \dot{R} - P(t) \]  

\( \text{Eq. 4.27} \)}
where $R$ represents the radius of the bubble, $p_{g0}$ the initial gas pressure inside the bubble, $p_v$ the vapour pressure, $\chi$ the polytrophic gas index, $S_p$ the stiffness of the shell and $\delta_i$ represents the total dampening.

### 4.2.4 Extinction cross-section

The attenuation of UCA is defined as the extinction cross-section ($\sigma_e$), which is based on the scattering of US ($\sigma_s$) and absorption of acoustic energy ($\sigma_a$) due to UCA bubbles, and is defined as

$$\sigma_e = \sigma_s + \sigma_a \quad \text{Eq. 4.28}$$

The ratio of the incident US scattered by the UCA bubbles is referred to as the scattering cross-section ($\sigma_s$) and is defined as [2]

$$\sigma_s = \frac{4 \cdot \pi \cdot R^2}{\left( f^2 \cdot f^{-2} - 1 \right)^2 + \delta_{tot}^2}, \quad \text{Eq. 4.29}$$

with $\delta_{tot}$ representing the total dampening which is based on three dampening terms, dampening due to re-radiation ($\delta_{rad}$), viscosity of surrounding tissue ($\delta_{vis}$) and thermal effects ($\delta_{th}$). The total dampening is defined as

$$\delta_{tot} = \delta_{rad} + \delta_{vis} + \delta_{th}, \quad \text{Eq. 4.30a}$$

$$\delta_{rad} = k \cdot R, \quad \text{Eq. 4.30b}$$

$$\delta_{vis} = 4 \cdot \mu \cdot \left( \rho \cdot \omega \cdot R^2 \right)^{-1}, \quad \text{Eq. 4.30c}$$

$$\delta_{th} = B(\omega, R) \cdot f_r^2 \cdot f^{-2}. \quad \text{Eq. 4.30d}$$

Here $\mu$ represents the viscosity of the surrounding tissue and $k$ the wave number. All three dampening terms are maximal for $f = f_r$. The bubble radius dependent term $B(\omega, R)$ is defined as [2]

$$B(\omega, R) = 3(\gamma - 1) \left[ \frac{X(\sinh X + \sin X) - 2(\cosh X - \cos X)}{X^2(\cosh X - \cos X) + 3(\gamma - 1)X(\sinh X - \sin X)} \right], \quad \text{Eq. 4.31}$$

with

$$X = R \sqrt{2 \cdot \omega \cdot \rho_g \cdot C_p \cdot K^{-1}}. \quad \text{Eq. 4.32}$$

The term $\rho_g$ here represents the density of the encapsulated gas.

The US energy absorbed by an UCA bubble and converted into heat is defined by the absorption cross-section ($\sigma_a$) as

$$\sigma_a = \sigma_s \left( \frac{\delta_{tot}}{\delta_{tot}} - 1 \right), \quad \text{Eq. 4.33}$$

The attenuation property of tissue perfused with UCA depends strongly on the pressure of the US wave [7, 30]. The pressure dependent attenuation is caused by the pressure dependency of $\delta_{tot}$, due to the pressure dependency of $R$ as shown in
the previous section, and enforced by the frequency dependency of the attenuation of tissue.

During echocardiography, US travels through a significant depth of tissue containing varying concentrations of UCA. As a result the attenuation at a certain depth is a function of the attenuation of UCA and tissue along the entire path. The attenuation along the path causes the US pressure to drop. As a result, attenuation at larger depth increases.

4.3 Nonlinear propagation models

The strong nonlinearity of an UCA together with the interactions between the different nonlinear properties of tissue and UCA results in the need for a nonlinear model to describe the distortion of US under influence of an UCA dilution.

The developed models help to understand and predict the nonlinear US distortion and comparing different models gives information on the importance of several nonlinear properties.

In this paragraph three different models are described, all based on the physics of the interaction between US and the nonlinear properties of UCA and medium. The first two models described in section 4.3.1 interpret tissue and UCA as one nonlinear medium for the US and are derived from the Burger's equation. The third model incorporates the Rayleigh Plesset equation into the Burger's equation to incorporate the bubble dynamics in the model and is described in section 4.3.2

4.3.1 Nonlinear medium

The first two nonlinear models used to model the nonlinear US distortion due to UCA are based on the Burger's equation. These models include nonlinearity up to the second order and attenuation. Both models look at the combination of tissue and UCA as one nonlinear medium. The nonlinearity from the UCA can be extracted from the model due to the small nonlinearity of tissue.

Before going into the details of the models, the Burger's equation is derived. The Burger's equation is based on three physical laws, which the propagating US must satisfy. One is the equation of state, which describes the relation between pressure and density and is already derived in section 4.1.1. Furthermore the propagating US has to satisfy the continuity equation, described in section 4.3.1.2 and the momentum equation, described in section 4.3.1.3. The solution of the Burger's equation and the two models derived from this are discussed in section 4.3.1.4 till 4.3.1.6. The fitting of the measurement data and the derivation of the coefficients $\alpha$ and $\beta$ are discussed in section 4.3.1.7.

4.3.1.1 Equation of state

The equation of state describes the relation between the density variation ($\rho'$) and the pressure variation ($P'$) and is already derived in section 4.1.1 for convenience equation 4.19 is repeated here:

$$P' = c_0^2 \rho' + \left( B \cdot (2A)^{-1} \cdot c_0^2 \cdot \rho_0^{-1} \right) \rho'^2 - K \cdot \left( \rho_0 \cdot c_0^2 \right)^{-1} \left( C_v^{-1} - C_p^{-1} \right) \frac{\partial \rho'}{\partial t}. \quad \text{Eq. 4.19}$$

Just like for the nonlinearity, it is assumed for all further derivations, that all terms of order three or higher are small compared to the second order and therefore can be discarded. Due to this assumption, all second order terms can be substituted to another variable using a relation of the first order, because the error made is of the third order and can thus be discarded.
For the further derivation of the Burger's equation it is necessary to rewrite equation 4.19 as \( \rho' = \rho'(P') \). The first order relation between \( \rho' \) and \( P' \) needed for this substitution is shown in equation 4.2

\[
\rho' = P' \cdot c_0^2.
\]

Eq. 4.34

Combining equations 4.19 with equation 4.34 leads to the following equation of state:

\[
\rho' = P' \cdot c_0^2 - (\lambda - 1) \cdot \left( 2 \cdot \rho_0 \cdot c_0^4 \right)^{-1} \cdot P'' - K \cdot \left( \rho_0 \cdot c_0^4 \right)^{-1} \left( C_\rho^{-1} - C_p^{-1} \right) \frac{\partial P'}{\partial t}.
\]

Eq. 4.35

4.3.1.2 Continuity equation

The second physical law that has to be satisfied is the continuity equation, defined as \([2, 11, 23, 25]\)

\[
\frac{D \rho}{D t} + \rho \cdot \nabla v = 0,
\]

Eq. 4.36

with \( v \) representing the particle speed. Notice that \( \frac{D}{D t} \) does not represent the normal time derivative, instead it represents the material time derivative which is defined as

\[
\frac{D}{D t} = \frac{\partial}{\partial t} + v \cdot \nabla
\]

Eq. 4.37

The continuity equation shown in equation 4.36 describes the variations of the density of a medium in time under the influence of \( v \) (and thus \( P' \)). The relation ensures that the conservation of mass laws are fulfilled.

Combining equations 4.36 with the definition of the material time derivative, equation 4.37, gives

\[
\frac{\partial \rho'}{\partial t} + \rho_0 \cdot \nabla v = -\rho' \cdot \nabla v - v \cdot \nabla \rho'.
\]

Eq. 4.38

Equation 4.38 can be used together with equation of state and the momentum equation to derive the Burger's differential equation.

4.3.1.3 Momentum equation

The third physical law that has to be satisfied is the momentum equation, defined as \([2, 11, 23, 25]\)

\[
\rho \frac{D v}{D t} + \nabla P = \mu \cdot \nabla^2 v + \left( \eta_B + \frac{1}{3} \eta \right) \cdot \nabla (\nabla v),
\]

Eq. 4.39

where \( \eta \) and \( \eta_B \) denote respectively the thermal and bulk viscosity of the medium.

The momentum equation describes the concentration of momentum for the influence of US pressure on a medium.

Equation 4.39 can be rewritten using the definitions \([2]\):

\[
\nabla (\nabla v) = \nabla^2 v + \nabla \times \nabla \times v,
\]

Eq. 4.40a

\[
(v \cdot \nabla) v = \frac{1}{2} \nabla^2 v - v \times \nabla \times v,
\]

Eq. 4.40b

and the relation between the time and displacement derivative for a plane progressive wave
Combining the definitions from equation 4.40 and 4.41 with the momentum equation, and discarding the terms \((\eta_v + \frac{4}{3} \eta) \cdot \nabla \times \nabla \times v \) and \(\rho_v \cdot \nabla \times \nabla \times v\), because they are small compared to other second order terms [2], results in a momentum equation of the form

\[
\rho_0 \frac{\partial v}{\partial t} + \nabla P' = -\rho' \frac{\partial v}{\partial t} - \frac{1}{2} \rho_0 \cdot \nabla^2 v + \left(\eta_v + \frac{4}{3} \eta\right) \cdot \nabla^2 v
\]

**Eq. 4.42**

### 4.3.1.4 Burger’s equation

For the derivation of the Burger’s equation the equation of state, continuity equation and momentum equation are combined into one differential equation. To this end equations 4.38 and 4.42 are rewritten as a function of \(P'\), to be able to combine them with the equation of state, using the first order relation between \(v\) and \(P'\),

\[
v = c_0 \cdot \rho_0^{-1} \cdot \rho' = (c_0 \cdot \rho_0)^{-1} P'.
\]

**Eq. 4.43**

This results in

\[
\frac{\partial P'}{\partial t} + \rho_0 \cdot \nabla v = \left(\rho_0 \cdot c_0^4\right)^{-1} \frac{\partial P'^2}{\partial t} + c_0^2 \cdot \frac{\partial L}{\partial t},
\]

**Eq. 4.44**

\[
\rho_0 \frac{\partial v}{\partial t} + \nabla P' = \left(\rho_0 c_0^2\right)^{-1} \left(\mu_\theta + \frac{4}{3} \mu\right) \nabla \frac{\partial P'}{\partial t} - \nabla L.
\]

**Eq. 4.45**

With \(L\) representing the second order Lagrangian density which is defined as

\[
L = \frac{1}{2} \rho_0 \cdot v^2 - P'^2 \cdot \left(2 \cdot \rho_0 \cdot c_0^2\right)^{-1}.
\]

**Eq. 4.46**

For a plane progressive wave the Lagrangian density is zero, because the second order assumption makes it possible to write \(P'^2\) as \(v^2\) using the first order relation \(P' = c_0 \cdot \rho_0 \cdot v\). This assumption only holds for distances much larger than one wavelength [2].

Now equations 4.40, 4.44 and 4.45 can be combined to one differential equation. For this the time derivative of equation 4.44 is combined with equation 4.45 multiplied by \(\nabla\), equating the terms \(\rho_0 \cdot \nabla \frac{\partial v}{\partial t}\). The equation of state (equation 4.40) can be used to eliminate \(\rho'\). The differential equation now becomes

\[
\left(\nabla^2 - c_0^{-2} \cdot \frac{\partial^2}{\partial t^2}\right) P' + \delta \cdot c_0^{-4} \frac{\partial^3 P'}{\partial t^3} = -\beta \cdot \left(\rho_0 \cdot c_0^4\right)^{-1} \frac{\partial P'^2}{\partial t^2} - \left(\nabla^2 + c_0^{-2} \cdot \frac{\partial^2}{\partial t^2}\right) L,
\]

**Eq. 4.47**

with

\[
\delta = \rho_0^{-1} \cdot \left(\frac{4}{3} \mu + \mu_\theta\right) + K \cdot \rho_0^{-1}\left(C_v^{-1} - C_p^{-1}\right),
\]

**Eq. 4.48**

representing the diffusivity of the US and the coefficient of nonlinearity defined as
\[ \beta = \frac{\gamma - 1}{2} \]  
Eq. 4.49

The assumption of a plane progressive wave gives that \( L = 0 \). This is true for the application of contrast echocardiography and thus the differential equation becomes

\[ \left( \nabla^2 - c_0^{-2} \frac{\partial^2}{\partial t^2} \right) P' + \delta \cdot c_0^{-4} \frac{\partial^3 P'}{\partial t^3} = -\beta \cdot \left( \rho_0 \cdot c_0^4 \right)^{-1} \frac{\partial P'^2}{\partial t^2}. \]  
Eq. 4.50

Reducing the three-dimensional differential equation of equation 4.50 to one dimension gives

\[ \left( \frac{\partial^2}{\partial x^2} - c_0^{-2} \frac{\partial^2}{\partial t^2} \right) P' + \delta \cdot c_0^{-4} \frac{\partial^3 P'}{\partial t^3} = -\beta \cdot \left( \rho_0 \cdot c_0^4 \right)^{-1} \frac{\partial P'^2}{\partial t^2}. \]  
Eq. 4.51

Due to the slow variations of attenuation and nonlinearity as a function of distance it is possible to translate the differential equation to a delayed time. Due to this translation only derivatives of the first and second order remain. For the translation a new time unit \( \tau \) is introduced defined as

\[ \tau = t - x \cdot c_0^{-1}. \]  
Eq. 4.52a

\[ x_1 = x, \]  
Eq. 4.52b

with \( x_1 \) referred to as the slow scale displacement. Now the derivatives of time and distance become

\[ \frac{\partial}{\partial x} = \frac{\partial}{\partial x_1} - c_0^{-1} \cdot \frac{\partial}{\partial \tau}, \]  
Eq. 4.53a

\[ \frac{\partial}{\partial t} = \frac{\partial}{\partial \tau}, \]  
Eq. 4.53b

and the differential equation becomes

\[ 2 \cdot c_0^{-3} \frac{\partial}{\partial \tau} \frac{\partial^2 P'}{\partial x_1 \partial \tau} + \delta \cdot c_0^{-2} \cdot \frac{\partial^3 P'}{\partial x_1 \partial \tau^2} = -\beta \cdot \left( \rho_0 \cdot c_0^4 \right)^{-1} \frac{\partial P'^2}{\partial \tau^2}. \]  
Eq. 4.54

After reinstalling the physical coordinate \( x \) in place of \( x_1 \), the differential equation describing the distortion of the US due to the nonlinear medium is in the form called the Burger’s equation, which is described as

\[ \frac{\partial P'}{\partial x} + \frac{\sqrt{2} \cdot c_0^{-3}}{2} \cdot \frac{\partial^2 P'}{\partial x \partial \tau} = -\beta \cdot P' \cdot \left( \rho_0 \cdot c_0^4 \right)^{-1} \frac{\partial P'}{\partial \tau}. \]  
Eq. 4.55

In the case of a one frequency sinusoidal US signal, the exact result of the Burger's differential equation is derived in [2] and described by the following relation for \( P' \):

\[ P' = P_0 \cdot \frac{\int_{-\infty}^{\infty} \int_{-\infty}^{\infty} e^{\beta \cdot P_0 \cdot (\rho_0 \cdot c_0^4)^{-1} \int_{-\infty}^{\infty} \sin(\omega \cdot \tau') \cdot d\tau'} \cdot e^{-\delta^2 (\tau-\tau')^2 (2 \cdot x_0 \cdot \delta)^{-1}} \cdot d\tau'}{\int_{-\infty}^{\infty} e^{\beta \cdot P_0 \cdot (\rho_0 \cdot c_0^4)^{-1} \int_{-\infty}^{\infty} \sin(\omega \cdot \tau') \cdot d\tau'} \cdot e^{-\delta^2 (\tau-\tau')^2 (2 \cdot x_0 \cdot \delta)^{-1}} \cdot d\tau'}. \]  
Eq. 4.56
Equation 4.55 has only an exact solution if $\beta$ or $\alpha$ (equation 4.21) is zero, else the solution has to be approximated. For $\alpha = 0$ the exact solution is [2]

$$P' = P_0 \cdot \sin \left( \omega \cdot \tau + \omega \cdot \beta \cdot x \cdot \left( \rho_0 \cdot c_0^3 \right)^{1/2} \right),$$

Eq. 4.57

while for $\beta = 0$ the exact solution is [11]

$$P' = P_0 \cdot e^{-a \cdot x} \sin(\omega \cdot \tau).$$

Eq. 4.58

4.3.1.5 Simple nonlinear medium model

A first simple model for the propagation of US through a nonlinear medium is based on the exact solutions for the cases $\alpha = 0$ and $\beta = 0$, shown in equations 4.57 and 4.58. If assumed that the interaction between medium and US is almost fully determined by the nonlinearity of the medium an adjusted version of equation 4.57, including a simple attenuation term $(a)$, can be used to model the US distortion. The model is then given as

$$P' = P_0 \cdot a \cdot \sin \left( \omega \cdot \tau + \omega \cdot \beta \cdot x \cdot \left( \rho_0 \cdot c_0^3 \right)^{1/2} \right).$$

Eq. 4.59

4.3.1.6 Physical nonlinear medium model

A second model for the propagation of US through a nonlinear medium is based on the approximate solutions for the Burger’s equation, leaving more room for the interaction between medium nonlinearity and attenuation.

There are two approximations available for equation 4.55, one for a Gol’dberg number (equation 4.20) $\Gamma < 1$ (attenuation is more dominant then the nonlinearity) the other for $\Gamma \gg 1$ (nonlinearity is more dominant then attenuation). For the approximations it is necessary to rewrite equation 4.56 as shown by Blackstock et al. [2] to an equation of the form

$$P'(x, \tau) = P_0 \cdot \sum_{n=1}^{\infty} B_n(x) \sin(n \cdot \omega \cdot \tau).$$

Eq. 4.60

For $B_n$, which represents the spectral amplitude of the $n^{th}$ harmonic, no exact expressions are available. The expressions for $B_n$ have to be approximated.

In case $\Gamma < 1$, the spectral amplitudes till the third order can be expressed as [4]

$$B_1 = e^{-a \cdot x} - \frac{1}{32} \cdot \Gamma^2 \cdot e^{-a \cdot x} \cdot (1 - e^{-2a \cdot x}) + O(\Gamma^4),$$

Eq. 4.61a

$$B_2 = \frac{1}{4} \cdot \Gamma \cdot (e^{-2a \cdot x} - e^{-4a \cdot x}) + O(\Gamma^3),$$

Eq. 4.61b

$$B_3 = \frac{1}{32} \cdot \Gamma^2 \cdot (2e^{-3a \cdot x} - 3e^{-5a \cdot x} + e^{-9a \cdot x}) + O(\Gamma^4).$$

Eq. 4.61c

In case $\Gamma \gg 1$ the spectral amplitudes can be expressed as [2]

$$B_n = \frac{2 \cdot \Gamma^{-1}}{\sinh(n \cdot (1 + \alpha) \cdot \Gamma^{-1})}.$$

Eq. 4.62

4.3.1.7 Model parameter estimation.

The simple nonlinear medium model has two parameters which need to be initialised to fit the model on the measurements. The simple attenuation term $a$ is initialised...
using the ratio between maximum amplitude of the measurement and $P_0$. The coefficient of nonlinearity $\beta$ is initialised using its definition, shown in equation 4.5. The spectral amplitudes used in equation 4.5 can be approximated using the square root of the measured spectral powers.

The physical nonlinear medium model has the coefficients $B_n$ which need to be initialised to fit the model on the measurements. Because the parameter $B_n$ represents the spectral amplitude of the $n^{th}$ harmonic the fit of the model on the measurement can be initialized using the square root of the measured spectral powers of the harmonics.

The parameters of interest $\alpha$ and $\beta$ can be calculated using the fitted $B_n$ and the expressions for $B_n$ from the equations 4.61 and 4.62. The attenuation $\alpha$ can directly be derived from the definitions for $B_n$ and the nonlinearity $\beta$ is derived using the definition for $\Gamma$. It is assumed that the calculated $\alpha$ and $\beta$ represent the attenuation and nonlinearity from the UCA, because of the low attenuation and nonlinearity of water.

### 4.3.2 Bubble dynamics model

The bubble dynamics model is just like the models derived in the previous paragraph based on the Burger’s equation and includes the bubble dynamics in the model. The normal time Burger’s equation from equation 5.50 is used. The following derivations show how the bubble dynamics are included in the Burger’s differential equation.

In a mixture of UCA and tissue the density of the mixture is determined by

$$\rho_0 = N \cdot V_0 \cdot \rho_{g0} + (1 - N \cdot V_0) \cdot \rho_{t0}, \hspace{1cm} \text{Eq. 4.63}$$

with $N$ the number of UCA bubbles per unit volume, $V_0$ the volume an UCA bubble occupies in unperturbed state, $\rho_{g0}$ the unperturbed gas density and $\rho_{t0}$ the unperturbed medium density. When US passes through the UCA mixture, the density ratio becomes [2]

$$\rho_0 \cdot \rho^{-1} = N \cdot V + (1 - N \cdot V_0) \cdot \rho_{t0} \cdot \rho^{-1}, \hspace{1cm} \text{Eq. 4.64}$$

with $V = V_0 + V'$ being the total volume including the volume change due to bubble dynamics $(N \cdot V_0 \cdot \rho_{g0} \cdot \rho^{-1} = N \cdot V)$.

For small UCA concentrations $(NV_0 < 1)$, as is the case for contrast echocardiography, the approximation $\rho_0 \approx \rho_{t0} >> \rho_{g0}$ may be used to approximate equation 4.64 as [2]

$$\rho_0 \cdot \rho_0^{-1} = \rho_0 \cdot \left(\rho_{g0}^2 \right)^{-1} \approx N \cdot V'. \hspace{1cm} \text{Eq. 4.65}$$

Combining equation 4.65 with the continuity and momentum equation results in a Burger’s equation as

$$\nabla^2 P' - \rho_0^{-2} \frac{\partial^2 P'}{\partial t^2} = -\rho_0 \cdot N \cdot \frac{\partial^3 V'}{\partial t^2}. \hspace{1cm} \text{Eq. 4.66}$$

Here the attenuation and nonlinearity from the medium are assumed negligible as with the previous model and all bubbles are assumed to have the same radius. This
last assumption is not true, because all UCA have a bubble size distribution, but is needed for the solvability of the model.

To solve the differential equation from equation 4.66 it is necessary to know the relation between the pressure variation \( P' \) and the volume variation \( V' \). This relation can be determined using the Rayleigh-Plesset equation [2],

\[
R \cdot \dot{R} + \frac{3}{2} \ddot{R} + 4 \cdot \varphi \cdot \dot{R} \cdot R^{-1} = (P_g - P) \cdot \rho_0^{-1}, \tag{4.67}
\]

to model the dynamic response of a single bubble. Combining the Rayleigh-Plesset equation with the volume equation for a spherical bubble

\[
V = \frac{4\pi}{3} \cdot R^3, \tag{4.68}
\]

and the adiabatic gas law

\[
P_g \cdot \rho_0^{-1} = (V_o \cdot V^1)^2, \tag{4.69}
\]

permits the expansion of Rayleigh-Plesset equation to be expressed in terms of \( V \) as [2]

\[
\ddot{V} + \delta_{ua} \cdot \omega_0 \cdot \dot{V} + \omega_0^2 \cdot V + \mu \cdot P' = (A + 1) \cdot \omega_0^2 \cdot (2 \cdot V_0)^{-1} \cdot V^2 + \sqrt[6]{V_0} \cdot (2 \cdot V \cdot \dot{V} - \dot{V}^2). \tag{4.70}
\]

Here

\[
\omega_0^2 = 3 \cdot \gamma \cdot P_0 \cdot \left( \rho_0 \cdot R_0^2 \right)^{-1} \tag{4.71}
\]

represents the resonance frequency of the UCA bubble. Equation 4.70 now gives the needed relation between pressure variation and volume variation to solve the differential equation 4.66. This can be done using the method of successive approximations. First approximating the solution using only \( \tilde{P}_1 \) and \( V_1 \) then expanding the approximate solution using the found \( \tilde{P}_1 \), \( V_1 \) and including \( P_2 \) and \( V_2 \). The pressure and volume relation till the second order then looks like

\[
P' = \frac{1}{2} \left( P_1 \cdot e^{i \omega_0 t} + P_2 \cdot e^{i 2 \omega_0 t} \right), \tag{4.72a}
\]

\[
V' = \frac{1}{2} \left( V_1 e^{i \omega_0 t} + V_2 e^{i 2 \omega_0 t} \right). \tag{4.72b}
\]

To derive the attenuation, dispersion and nonlinearity from the model shown in the equations 4.72 it is necessary to derive \( P_1 \) and \( P_2 \).

Substitution of equations 4.72 into equations 4.66 and 4.70 gives

\[
\left( \nabla^2 + \omega^2 \cdot c_0^{-2} \right) P_1 = \rho_0 \cdot N \cdot \omega^2 \cdot V_1, \tag{4.73}
\]

\[
\left( \omega_0^2 - \omega^2 + j \cdot \delta_{ua} \cdot \omega_0 \cdot \omega \right) V_1 = -\mu \cdot P_1. \tag{4.74}
\]

Eliminating \( V_1 \) from both equations by combining them gives one differential equation for the fundamental amplitude \( P_1 \):

\[
\left( \nabla^2 + \omega^2 \cdot \tilde{c}_1^{-2} \right) \cdot P_1 = 0. \tag{4.75}
\]

Here \( \tilde{c}_1 \) is defined as [2]
\[ c_0^2 \cdot \tilde{c}_n^{-2} = 1 + \frac{N \cdot V_0 \cdot \rho_0 \cdot c_0^2 \cdot (\gamma \cdot P_2)^{-1}}{1 - n^2 \cdot \omega_2^2 + j \cdot \delta_{vis} \cdot n \cdot \omega \cdot \omega_0^{-1}} \cdot \quad \text{Eq. 4.76} \]

Now the dispersion and attenuation of the medium are determined as [2]
\[ c_n(\omega) = \text{Re}[\tilde{c}_n^{-1}]^{-1}, \quad \text{Eq. 4.77} \]
\[ \alpha_n(\omega) = -n \cdot \omega \cdot \text{Im}[\tilde{c}_n^{-1}], \quad \text{Eq. 4.78} \]

Substitution of equations 4.72 into equations 4.66 and 4.70 gives
\[ \left( \nabla^2 + 4 \cdot \omega_2^2 \cdot \tilde{c}_2^{-2} \right) P_2 = 4 \cdot \rho_0 \cdot N \cdot \omega_2^2 \cdot V_2, \quad \text{Eq. 4.79a} \]
\[ \left( \omega_0^2 - 4 \cdot \omega_2^2 \cdot j \cdot 2 \cdot \delta_{vis} \cdot \omega_0 \cdot \omega \right) V_2 = -\mu \cdot P_2 + \frac{1}{2} \cdot \left( a - 3 \cdot b \cdot \omega^2 \right) \cdot V_1^2. \quad \text{Eq. 4.79b} \]

Eliminating \( V_2 \) from both equations by combining them gives one differential equation for the second harmonic amplitude \( P_2 \) :
\[ \left( \nabla^2 + 4 \cdot \omega_2^2 \cdot \tilde{c}_2^{-2} \right) P_2 = \beta(\omega) \cdot 2 \cdot \omega^2 \left( \rho_0 \cdot c_0^4 \right)^{-1} P_1^2. \quad \text{Eq. 4.80} \]

### 4.3.2.1 Model parameter estimation

The model derived in equations 4.72 for the nonlinear distortion of US due to bubble dynamics has the parameters \( P_1 \) and \( P_2 \) which have to be adapted to the measured distortion. Because the parameter \( P_n \) is related to the spectral amplitude of the \( n \)th harmonic the fit of the model on the measurement can be initialized using the square root of the measured spectral powers from the harmonics.

After the derivation of \( P_1 \) and \( P_2 \), the values for \( \delta_{vis} \) and \( \gamma \), which are the only two unknowns present in the differential equations, can be derived by solving both differential equations 4.74 and 4.80.

The attenuation and dispersion for fundamental and second harmonic frequency can then be calculated using equations 4.77 and 4.78. The nonlinearity parameter \( \beta \) can be calculated as [2]:
\[ \beta(\omega) = \frac{\eta \cdot C^2 \left( \gamma + 1 - \omega^2 \cdot \omega_0^{-1} \right)}{2 \cdot \left( 1 - 4 \cdot \omega_0^2 - j \cdot 2 \cdot \delta_{vis} \cdot \omega_0 \cdot \omega \right) \left( 1 - \omega^2 \cdot \omega_0^{-2} + j \cdot \delta_{vis} \cdot \omega_0 \cdot \omega \right)^2}. \quad \text{Eq. 4.81} \]

### 4.4 Nonlinear distortion measurements

The use of indicator dilution principles requires knowledge of the course of nonlinear US distortion for increasing UCA concentrations, to show the consequences for contrast imaging and quantification, as well as the dominant nonlinear properties.

Section 4.4.1 gives a description of the measurement set-up used to determine the nonlinear US distortion. The experimental results are shown in section 4.4.3 and the chapter ends with the conclusions.

#### 4.4.1 Measurement set-up

The influence of the Luminy concentration on the nonlinear US distortion is measured using several Luminy concentrations in the range used for contrast echographic quantifications. The results from the calibrations, shown in paragraph 3.2, show that the concentration range should be limited till concentrations below
19.5 µL/L. This is the range where a linear calibration was observed. The used concentrations and their preparation are described in appendix B.

For the measurements, a single element transducer (Olympus, A302S-SU immersion transducer) with a resonance frequency of 1 MHz is used. The measurements are performed for three frequencies, one below the resonance frequency of Luminity (1 MHz), one at its resonance frequency (1.5 MHz) and one above its resonance frequency (2 MHz).

A hydrophone (Onda HGL-0400, bandwidth 250 kHz -15 MHz) is used as receiver, receiving the US influenced by the Luminity dilution.

The latex tube, with a diameter of approximately 3.4 cm, with the Luminity dilution is placed with its centre 4.3 cm from the transducer, in the transducers focal zone. This is done to maximize the acoustic intensity at the Luminity dilution. To prevent the disturbance of the US signals by reflections between latex tube, transducer and hydrophone, the hydrophone is placed at a distance of 8.6 cm. This distance ensures that reflections reach the hydrophone after the US signal is detected.

![Diagram](image)

**Figure 4.5**: Measurements set-up nonlinear propagation of US through a Luminity dilution.

The backscatter from the surrounding measurement set-up is minimized by surrounding the latex tube filled with the Luminity dilution with acoustic absorbing material (sponges) as shown in figure 4.5. The measurement set-up is filled with water to match the acoustic impedance of the saline solution used for the Luminity dilutions and, by that, to minimize the backscatter due to a difference in acoustic impedance. Furthermore water is has good propagation properties for US. The measurement error due to air bubbles in water is minimized by filling the measurement set-up a day before use. By doing this, air bubbles added to the water due to the filling of the measurement set-up have time to be released from the water.

The destruction of Luminity bubbles is minimized by the use of a low MI of 0.1, like for the calibration measurements. The MI is measured in the focal zone of the transducer.

Before use, the latex tubes with the Luminity dilution is gently hand shaken for 10 seconds, to ensure a uniform mixing of the Luminity dilution.

The distortion measurements are performed using a sinusoidal wave of ten periods as shown in figure 4.6. The sinusoidal signal is made using a pulse generator (Agilent, 3322 OA) and amplified using a RF amplifier (ENI, 240L). The length of the sinusoidal wave is a compromise between the frequency resolution (a longer signal
gives a better frequency resolution in the frequency domain) and the bubble exposure time (a longer signal destroys more bubbles). The chosen length leads to a practical frequency resolution of 0.011 MHz (1 MHz measurement, 9 useful periods).

Figure 4.6: Sinusoidal wave used for nonlinear distortion measurements.

The sampling of the received signal is done using the maximum sample frequency of the A/D converter (National Instruments). This ensures that the Nyquist theorem is fulfilled and signals till the fourth harmonic can be analyzed for all used frequencies. Precise specifications of the measurement set-up are given in appendix B.

4.4.2 Analysis of the nonlinear distortion measurements

The analysis of the measured acoustic pressure is performed using Matlab (The Mathworks). Before analysis, the measured pressure signal is low pass filtered, removing unwanted frequencies above the fourth harmonic. The specifications of the low pass filter are given in appendix B. After low pass filtering, the US signal of interest is extracted from the measured acoustic signal using a Hamming window to minimize frequency leakage.

The US signal of interest is transformed to the frequency domain using a fast Fourier transform (FFT). The frequency response is used to determine the nonlinear US distortion due to Luminity by analyzing the spectral intensities from the harmonic frequency bands, including sub-, ultra- and super-harmonics, normalised with respect to the total integrated spectral intensity for each concentration. The bandwidth of the different harmonic frequency bands is determined by the cut-off frequency (frequency where the intensity drops 3 dB with respect to the maximum).

All analysis steps are also performed for a part of the measurement with no US signal present to determine the noise power in each harmonic band. This noise measurement is done to determine the signal-to-noise ratio (SNR).

Next to the frequency analysis, a time domain analysis of the distortion is done using the three nonlinear propagation models derived in paragraph 4.3.

The analyses of the measured acoustic pressure is expected to show an increase in attenuation for increasing Luminity concentrations. For concentrations above 13.5 μL/L, the measurements should show a strong increase in attenuation, as above 13.5 μL/L shadowing becomes noticeable. The measured nonlinearity is expected to increase for increasing Luminity concentrations. According to the second order assumption made in paragraph 4.1, the strongest increase in power fraction due to increasing nonlinearity should be found in the second-harmonic band. Other harmonics are expected to show a less strong increase.
4.4.3 Results of the nonlinear distortion measurements

The results show that the transducer cannot transmit a pure 2 MHz sinusoid, as shown in figure 4.7. The distortion causes a large variation in the MI during the 10 periods of the waveform and a higher noise level in relation to the 1 MHz and 1.5 MHz measurements. Due to this, the results from the measurements at 2 MHz are discarded. Individual measurement from the 1 MHz and 1.5 MHz measurements which are discarded, due to large differences in relation to other measurements, are shown in appendix C.

![Figure 4.7: Example of the nonlinear distortion for all signals sent at 2 MHz.](image)

The analyses of the noise power for different nonlinear distortion measurements shows that the noise power is not constant for varying concentrations. It was expected to see a constant noise power. This variation in noise power is present for all frequency bands analysed and is shown in figure 4.8. This variance in noise power reduces the SNR for low Luminity concentrations, as shown in appendix D.

The found variance in noise power is most probably caused by the change in quantisation range for increasing Luminity concentrations. These changes were done to adapt the quantisation range to the increasing attenuation and maximise the resolution for all measurements. Due to the use of a larger quantisation range for low Luminity concentrations also a larger quantisation step was used, resulting in a larger measured noise level.

![Figure 4.8: Noise power spectrum.](image)

Most analyzed frequency bands show a high SNR for all concentrations, showing the usability of these frequency bands for contrast imaging. This due to the low
vulnerability for noise artefacts. An exception is the super-harmonic band, which shows a low SNR for most concentrations. Ultra- and forth-harmonic frequency bands only show a low SNR for low Luminity concentrations.

The attenuation is expressed with respect to the measurement using a zero μL/L dilution. The results of the attenuation analyses only show shadowing for the measurements at 1 MHz. Here shadowing gets noticeable from 18 μL/L, as shown in figure 4.9. For the measurements at 1.5 MHz no shadowing is noticeable.

As expected, all measurements show a decrease in power fraction at the fundamental frequency band and the strongest increase in power fraction at the second harmonic frequency band, as shown in appendix E. Unexpected is the minimal increase in power fraction for sub- and ultra-harmonic frequency band at 1 MHz and the decrease in power fraction for the ultra-harmonic frequency band at 1.5 MHz. Also at here a more increase of power fraction was expected. All other analysed frequency bands show the expected increase in power fraction for increasing Luminity concentrations.

The initialisation of the attenuation and nonlinearity coefficient estimated by the simple model derived in section 4.3.1.5 show a good resemblance between the initialised and fitted attenuation coefficient and a large deviation between the initialised and fitted nonlinearity coefficient. The initialisation of the spectral amplitudes for the fit of the Burger's model, derived in section 4.3.1.6, show a good resemblance with the fitted spectral amplitudes, as shown in appendix F.

The found attenuation and nonlinearity coefficients for all models are shown in appendix G. The simple model shows a noisy increase of the attenuation coefficient and a linear increase of the nonlinearity coefficient for increasing Luminity concentrations. The found nonlinearity coefficients are outside the range of thousand times the nonlinearity coefficient of tissue. The Burger's model for a Gol'dberg number >> 1 (nonlinearity stronger than attenuation), shows a large deviation for the attenuation and nonlinearity coefficient with respect to the Burger's model for Γ < 1 for concentrations below 3.75 μL/L. In this region Γ < 1 and this shows that the model for Γ >> 1 only holds for higher concentrations where Γ > 1. Above 3.75 μL/L the values for the attenuation and nonlinearity coefficient are close for both models.

The determination coefficients, shown in table 4.1, of the fit of the different models on the measurements show a good fit for all models in the entire concentration range. The use of a third order version of the Burger's model derived in paragraph 4.3.1 did
not show any significant improvements for concentrations till 13.5 \( \mu \text{L/L} \), as shown in table 4.1. Only for 18 \( \mu \text{L/L} \) small improvements where found (mean improvement of \( r^2 \) of 0.01, i.e. 1%).

No results were obtained for the bubble dynamic model described in section 4.3.2. This is due to the difficulties in extracting the attenuation and nonlinearity coefficients form the model fit.

Table 4.1 : Determination coefficients for the different measurements using a simple propagation model and a second and third order version of the Burger’s propagation model.

<table>
<thead>
<tr>
<th>Concentration (( \mu \text{L/L} ))</th>
<th>1 MHz</th>
<th>1.5 MHz</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Simple</td>
<td>2(^{nd}) order</td>
</tr>
<tr>
<td>0</td>
<td>( r^2=0.997 )</td>
<td>( r^2=0.997 )</td>
</tr>
<tr>
<td>0.375</td>
<td>( r^2=0.998 )</td>
<td>( r^2=0.998 )</td>
</tr>
<tr>
<td>0.75</td>
<td>( r^2=0.997 )</td>
<td>( r^2=0.997 )</td>
</tr>
<tr>
<td>1.05</td>
<td>( r^2=0.999 )</td>
<td>( r^2=0.999 )</td>
</tr>
<tr>
<td>1.5</td>
<td>( r^2=0.997 )</td>
<td>( r^2=0.997 )</td>
</tr>
<tr>
<td>2.25</td>
<td>( r^2=0.997 )</td>
<td>( r^2=0.997 )</td>
</tr>
<tr>
<td>3.75</td>
<td>( r^2=0.995 )</td>
<td>( r^2=0.996 )</td>
</tr>
<tr>
<td>5.25</td>
<td>( r^2=0.992 )</td>
<td>( r^2=0.994 )</td>
</tr>
<tr>
<td>7.5</td>
<td>( r^2=0.991 )</td>
<td>( r^2=0.995 )</td>
</tr>
<tr>
<td>10.5</td>
<td>( r^2=0.968 )</td>
<td>( r^2=0.985 )</td>
</tr>
<tr>
<td>13.5</td>
<td>( r^2=0.975 )</td>
<td>( r^2=0.992 )</td>
</tr>
<tr>
<td>18</td>
<td>( r^2=0.872 )</td>
<td>( r^2=0.945 )</td>
</tr>
</tbody>
</table>

4.4.4 Conclusions

Due to the found variation of noise power, the SNR for low concentrations are underestimated. Taking this into account no measurements are discarded due to an extremely low SNR, but it is shown that sub-, ultra and super harmonic frequency measurements are more sensitive to noise artefacts. This also holds for forth-harmonic frequency measurements using a low Luminity concentration, showing the drawback of higher harmonic frequencies for IDC determination.

The lack of shadowing for measurements at 1.5 MHz could be caused by a reduced Luminity concentration, because of earlier measurements. This would also explain the lower attenuation found for the measurements at 1.5 MHz. For future measurements less measurements should be performed using the same Luminity dilution, to show if this improves the results for 1.5 MHz.

As expected, the increase of spectral power is most significant for the second harmonic and less significant for other frequencies. The found unexpected low gain of spectral power for sub-, ultra and super-harmonic frequency bands, shown in appendix E, could be caused by the practical difference in MI in comparison to the calculated MI using the settings from appendix B. In practice the MI at the focal zone was measured to be 0.09 for all measurements. Further measurements with a slightly higher and lower MI would show if an increase in power fraction is present in these frequency bands and give information on the MI region where the Luminity contrast bubbles change from a linear response to a nonlinear response.

The agreement between the fitted spectral amplitudes found after fitting the physical Burger's model from section 4.3.1.6 to the measurements and the values used to initialize the model, as shown in appendix F, show the usefulness of the square root of the spectral power to initialize the model fitting. The large difference between the initialisation of the nonlinearity coefficient and the found nonlinearity coefficient after
the model fit shows the drawback of the simple model from section 4.3.1.5 and should be addressed before further research is done.

The small improvement of accuracy achieved with the use of third order versions of the Burger’s model shows that the assumptions made, that a second order model is strong enough to model the nonlinear behaviour and all higher order terms can be discarded, is justified. This conclusion is enforced by the good fits found when using the second order model.

The determination coefficients of the model fits, shown in table 4.1, prove that all models perform well in modelling the nonlinear US distortion. The use of the more physical and complex Burger’s model gives only a small increase of the determination coefficients. Although the simple model shows a good fit with a low complexity, the physical Burger’s model is preferred. This due to the bad initialisation of the nonlinearity and the extreme high values found for the nonlinearity coefficient (outside the expected range) when using the simple model. Only if these issues can be addressed, the simple model could be preferred.

The attenuation and nonlinearity coefficient till a concentration of 3.75 μL/L are best modelled using the physical Burgers model for a Gol’dberg number < 1. For concentrations higher than 3.75 μL/L the Gol’dberg number becomes slightly larger than one, but stays relatively close to one (max \( \Gamma = 7 \)). Due to the small Gol’dberg number the model for \( \Gamma < 1 \) can be used for the entire range of adopted Luminity concentrations. Further research could show if a weighted use of both models for concentrations above 3.75 μL/L could improve the determination of attenuation and nonlinearity.

The results from the different measurements did not show the resonance behaviour at a frequency of 1.5 MHz as was expected. New literature from De Jong et al [6] shows that the resonance frequency for Luminity is higher than 1.5 MHz. New measurements should be performed using higher frequencies.
5 Imaging implications

The basic imaging mode for echography is fundamental imaging. Here the transmitted and received frequencies are the same, which allows the use of relative simple and cheap transducers. When using fundamental imaging with contrast echocardiography, only the linear influence of the UCA bubble is seen by the US scanner. Because this linear response does not differ significantly from the linear response of tissue, the resulting CTR is low.

Several imaging methods have been developed to improve the CTR using modern wide band US probes and dedicated signal processing. The three most basic imaging methods for the improvement of the CTR are described in paragraph 5.1.

One negative implication for imaging of the nonlinear interaction between UCA and US was found during previous experiments: the backscatter, coming from tissue of the nonlinearly distorted US due to UCA. This negative imaging implication is discussed in paragraph 5.2.

Next to the experiments performed to determine the nonlinear US distortion, several measurements are done with a clinical US scanner testing the implications for contrast imaging. A description of the measurement set-ups and the results is given in paragraph 5.3. The chapter ends with the conclusions on the imaging implications.

5.1 Contrast echography

The analyses of nonlinear US distortion from chapter 4 shows an approximately linear interaction between tissue and US compared to the interaction between UCA and US. The focus on the nonlinear response removes the tissue response from the signal and thus improves the contrast detection and the CTR.

On method of focusing on the nonlinear US response due to UCA is measuring the harmonic frequencies generated by the nonlinear behaviour of the bubbles instead of the fundamental frequency. This method is called harmonic imaging and is described in section 5.1.1.

Next to harmonic imaging, solutions where introduced to remove the nonlinear US response due to UCA from the linear tissue response using fundamental imaging. The two most important methods using fundamental imaging are power modulation (PM) and phase modulation, which is also called pulse inversion. Both methods are described in the section 5.1.2.

5.1.1 Harmonic Imaging

Modern US probes are wide band, meaning that they are able to detect the harmonic backscatter due to the nonlinear response of the bubbles. Imaging technique based on this principle are called harmonic imaging.

As shown in chapter 4 the nonlinear US backscatter due to UCA causes a shift of signal power from the fundamental (sent) frequency to other frequencies, especially harmonics and sub- and ultra-harmonic frequencies. Modern US scanners have several harmonic modes present, sub-, ultra and second harmonic imaging as shown in figure 5.1. The amount of energy shifted to harmonic frequency depends on the properties of the UCA, the UCA concentration and the distance travelled through the UCA.

The acoustic backscatter intensity measured at the harmonic frequencies is lower than at the fundamental frequency, nevertheless the CTR is high due to the low signal energy coming from tissue at harmonic frequencies.
5.1.2 Power and phase modulation

The nonlinear US backscatter due to UCA can also be exploited using fundamental imaging techniques and signal processing. Two imaging techniques that do this are PM and phase modulation. The use of fundamental imaging makes it possible to use relative simple and cheap US probes. Examples of the pulse sequences send by both techniques are shown in figure 5.2.

PM uses three US bursts to eliminate the linear interaction between tissue and US. Two bursts have a half amplitude in regard to the third as shown in figure 5.2. Adding the two half amplitude bursts and subtracting the full amplitude burst filters the linear tissue response and leaves the nonlinear UCA response. This principle is based on simple mathematics, for a linear function \( f(x) \) holds \( f(x) + f(x) = f(2x) \). This relation is not true for a nonlinear function \( g(x) \), \( g(x) + g(x) \neq g(2x) \). Now it is simple to see that when a function has a linear and a nonlinear part, only the nonlinear part remains using PM. PM can also be implemented using two US pulses, one pulse with half amplitude with respect to the other. Now the half amplitude pulse is multiplied by two and the result is subtracted from the full amplitude pulse.

Phase modulation uses two US bursts to separate the linear tissue interaction from the nonlinear UCA interaction. The second burst is the inverse of the first one (a phase shift of \( \pi \)). When subtracting both pulses the linear responses are filtered and the nonlinear responses between UCA and US remain.

New techniques combine both power and phase modulation with several different pulse sequence schemes. Eckersley et al [9] shows that these combinations perform better than harmonic imaging, power modulation or phase modulation.
5.2 Backscatter by tissue of UCA generate nonlinearity

During previous measurements a non expected effect of the backscatter of harmonic signals by acoustic absorbing material (sponges) was noticed as shown in figure 5.3. Research in literature showed that only one article [3] mentions briefly this phenomena. When no UCA is present, the sponge shows very limited harmonic backscatrer, as expected. When the latex tube with the UCA dilution is placed between the sponges and the transducer, the sponges start reflecting (linearly) the harmonics generated by the UCA. This reflection of harmonics is the inverse of the shadowing effect shown in figure 2.2. Shadowing is caused by attenuation, blocking the US from the deeper tissue. The Backscatter by tissue of UCA distorted US (with a broader spectrum) is related to the linear backscatter of tissue. A consequence of this backscatter by tissue is a decrease of CTR.

Figure 5.3 : Backscatter by tissue of UCA generated nonlinearity with TCE ultra harmonic imaging. Left without contrast. Right with Luminity (18 μL/L).

5.3 Imaging measurements

Two different measurement set-ups are used to show the imaging implications of nonlinear US distortion due to UCA bubbles. First the implications for the different contrast detection modes are determined using the measurement set-up described in section 4.4.1. The adjustments to the measurement set-up are described in section 5.3.1. A second set of measurements by a tissue mimicking phantom is used to determine the backscatter from tissue of US nonlinearly distorted by UCA. This measurement set-up is described in section 5.3.3. The results from the imaging measurements are shown in sections 5.3.2 and 5.3.4, and the conclusions are given in section 5.3.5.

5.3.1 Contrast imaging set-up

The influence of nonlinear US distortion due to UCA on the contrast imaging methods is determined using the measurement set-up described in section 4.4.1.

The implications for harmonic imaging are determined using the frequency spectrum analyses from the ten period sinusoidal waveform used for the measurement of the nonlinear US distortion. The implications for PM are determined using two pulse sequences as shown in figure 5.4.
Figure 5.4: Pulse sequences used for determining the imaging implication for power modulation and phase modulation.

Both pulse sequences are built using three one-period sinusoidal pulses with a delay of thirteen $\mu$s between the different pulses. The first and the third pulse have a half amplitude with respect to the middle pulse. The difference between the two pulse sequences is a phase difference of $\pi$. The analyses of the imaging implications is done in the same way as for the nonlinear distortion measurements, described in section 4.4.2.

Four different applications of PM are analyzed: twice the first half amplitude pulse minus the full amplitude pulse, twice the last half amplitude pulse minus the full amplitude pulse and this for both phased signals.

The implications for contrast detection are determined using the power fractions of the different harmonics. A higher harmonic power fraction corresponds to a larger artefact as explained in section 5.2

5.3.2 Imaging implications

The implications for harmonic imaging are already shown in section 4.4.3. The increase in power fraction is strongest for the second harmonic frequency.

For the measurements at 1 MHz Sub-, Ultra-, Second-, Super and third harmonic frequency bands show the expected relation between power fraction and increasing Luminity concentrations as explained in the previous section. The highest increase of power fraction is found using Ultra- or Second-harmonic frequencies. The results for the measurements at 1.5 MHz show a more noisy relation between power fraction and Luminity concentration. Here Sub-, Ultra-, Second- and Third-harmonic frequencies show the expected relation between power fraction and increasing Luminity concentrations. The highest increase of power fraction is found using second harmonic frequencies, but here the increase is much lower than for the measurements at 1 MHz.

The results from the four different PM applications show the highest increase of power fraction for the positive phased signals, as shown in appendix I. For most measurements twice the last half amplitude pulse minus the full amplitude pulse showed the highest increase of power fraction.

The results for power modulation show a large amplitude difference between the two signals with a phase difference of $\pi$. Due to this difference it is not possible to filter...
the nonlinear contrast response from the measurements using phase modulation and all phase modulation results are discarded.

5.3.3 Backscatter measurement set-up

The goal of the backscatter measurements is to show and quantify the backscatter by tissue nonlinearity due to UCA for different contrast imaging modes. The quantification is then used to determine the consequences for imaging.

The range of Luminity concentrations is the same as for the nonlinear distortion measurements (appendix B), to allow the comparison of the results. For the measurement of the acoustic intensity, an Omniplane III (Philips Medical systems) TEE probe is used. The backscatter from the walls of the measurement set-up is minimized by covering the inside of the set-up with acoustic absorbing material, as shown in figure 5.5.

The different UCA dilutions are made following the calibration protocol shown in appendix A. The concentrations of the dilutions are expressed in μL/L and represent the amount of C₃F₈ gas in μL per litre saline. The concentrations used for the measurement are listed in appendix B and are made using a physiological saline solution (0.9%). The used volumes of saline have to be measured precisely, because any volume error causes an error in the concentration of the dilution of the same order as the volume error. Due to the small concentrations of interest, up to three dilution steps are necessary to reach the smallest concentrations. In these three dilution steps the concentration errors add up. Before the measurements are performed the latex tubes with the Luminity dilutions are gently shaken to ensure uniform mixing.

![Figure 5.5: Scheme of the set-up for backscatter measurements](image)

Just behind the latex tube with UCA a tissue phantom is placed (ATS Laboratories) to observe the backscatter by the tissue phantom of the nonlinear distorted US due to the Luminity dilution.

The destruction of UCA bubbles has to be minimized. To achieve this, all measurements are done with a low MI of 0.1 and the exposure to the US is kept short in time.

The calibrations are performed using a Sonos 5500 US scanners and three different imaging modes, TCE harmonic, TCE ultra-harmonic imaging and amplitude modulation. These modes are chosen to compare the results with the nonlinear distortion measurements.
The setting of the US machine and the measurement set-up are kept constant for all measurements.

The quantification of the measured acoustic intensity in the region of interest (ROI) is performed using Qlab (Philips Medical Systems). The analyses of the relation between the acoustic intensity and the UCA concentration is performed using Matlab (the Mathworks). The ROI for the measurement of the acoustic intensity is placed at the end of the latex tube with the Luminity dilution. For the measurement of the backscatter by tissue a second ROI is placed at the beginning of the tissue phantom, just behind the tube with the Luminity dilution. For the quantification of the backscatter by tissue a reference measurement is made determining the tissue phantom response in the ROI during the time where no contrast is present.

It is expected that the backscatter by tissue increases together with the acoustic intensity from the Luminity dilution for increasing concentrations. The backscatter by tissue is expected to be present with all contrast imaging modes. About the strength of the backscatter by tissue for different imaging mode no predictions can be made.

5.3.4 Results backscatter measurements

The results for the measurement of the backscatter by tissue show two regions with a different response.

In the first region, the lower concentration range, the nonlinear backscatter of the contrast is below the reference backscatter of the tissue phantom. In this region the backscatter of the tissue phantom shows an increase with respect to the reference level, as shown in figure 5.6.

In the second region, the higher concentration range, the nonlinear backscatter of the contrast is above the reference backscatter of the tissue phantom. In this region the backscatter of the tissue phantom increases directly with the contrast response, showing the negative effect on the CTR.

During the measurements an unexpected high contrast response was noticed for harmonic imaging and low Luminity concentrations.

The results, as shown in appendix H, show that the highest reduction of CTR is determined for ultra-harmonic imaging.

![Figure 5.6: Backscatter of nonlinear US by tissue phantom, ultra-harmonic imaging.](image)

5.3.5 Conclusions

The results for the different PM applications show that a positive phased signal, using twice the last half amplitude pulse minus the full amplitude pulse, and ultra- or
second harmonic imaging gives the highest increase of power fraction for increasing Luminity concentrations.

The noisy results for the measurements at 1.5 MHz and the low power could be caused by the bubble destruction due to earlier measurements with the same dilution. For further measurements it is recommended to use the dilutions for fewer measurements to see if this influences the results. Also more research on the bubble stability may help explain this.

For future measurements it is recommended to use two period pulses for PM measurements. This allows to reduce the amplitude difference between the two phased signals due to transitory states of the transducer. If this does not suffice, amplitude correction has to be implemented for future measurements.

The results for the backscatter by tissue, reported in appendix H, show its negative effect. The CTR, which is the important measure for the performance of a contrast detection modes, is reduced with a maximum of 60%. The unexpected high response for low Luminity concentrations and harmonic imaging makes the results from this measurement questionable. A new measurement using harmonic imaging is recommended to show if this measurement was disturbed.
6 Discussion and conclusions

As expected from previous results, all calibrations show a linear relation between acoustic backscatter intensity and Luminity concentration for concentrations from zero till shadowing occurs. The high determination coefficients found for the linearity, shown in table 3.1, prove a linear relation between acoustic backscatter intensity and Luminity concentration and the suitability of harmonic and ultra-harmonic imaging for the determination of an IDC. The best calibration result is found using harmonic imaging and an iE33 US scanner, because this calibration shows the highest linearity and the largest linear range. Since only one set of calibrations is performed, it is not possible to look at the improvement of the reproducibility using the new calibration method. Further calibrations are necessary and planned already to investigate reproducibility and to enforce the found results.

The results from the nonlinear distortion measurements show that although high harmonic frequencies (forth harmonic and higher) may have a larger CTR, they also have a lower SNR, especially for low UCA concentrations, making them less accurate due to the high sensitivity for noise. The results further show that sub-, ultra and super harmonic frequencies have a relative low SNR, making them less accurate due to the high sensitivity for noise. Although differences in SNR where found, no measurements where discarded due to an extreme low SNR.

As expected, the increase of spectral power is most significant for the second harmonic frequency band and less significant for other frequencies. The found unexpected low gain of spectral power for sub-, ultra and super-harmonic frequency bands, shown in appendix E, could be caused by the practical difference in MI in comparison to the calculated MI using the settings from appendix B. In practice the MI at the focal zone was measured to be 0.09 for all measurements. Further measurements with a slightly higher and lower MI would show if an increase in power fraction is present in these frequency bands and give information on the MI region where the Luminity contrast bubbles change from a linear response to a nonlinear response.

The agreement between the fitted spectral amplitudes found after fitting the physical Burger’s model from section 4.3.1.6 to the measurements and the values used to initialize the model, as shown in appendix F, shows the usefulness of the square root of the spectral power to initialize the model fitting. The large difference between the initialization of the nonlinearity coefficient and the found nonlinearity coefficient after the model fit shows the drawback of the simple model from section 4.3.1.5 and should be addressed before further research is done.

The small improvement of accuracy achieved using a third order version of the physical Burger’s model shows that the assumption made, that a second order model is accurate enough to model the nonlinear behaviour and higher order terms can be discarded, is justified. This conclusion is enforced by the good fits found using the second order model.

The determination coefficients of the model fits, shown in table 4.1, show that all models perform well in modelling the nonlinear US distortion. The use of a more physical and complex Burger’s model gives only a small increase of the determination coefficients. Although the simple model shows a good fit with a low complexity, the physical Burger’s model is preferred. This due to the bad initialization of the nonlinearity and the extreme high values found for the nonlinearity coefficient (outside the expected range) when using the simple model. Only if these issues can be addressed, the simple model is preferred.

The attenuation and nonlinearity coefficient till a concentration of 3.75 μL/L are best modelled using the physical Burgers model for a Gol'denberg number < 1. For
concentrations higher than 3.75 μL/L the Gol’dberg number slowly gets higher than one, but stays relative close to one (max $\Gamma = 7$). Due to the small Gol’dberg number the model for $\Gamma < 1$ can be used for the entire range of used Luminity concentrations. Further research could show if a weighted use of both models for concentrations above 3.75 μL/L could improve the determination of the attenuation and nonlinearity coefficient.

The results from the different measurements did not show the resonance behaviour at a frequency of 1.5 MHz as was expected. New literature from de Jong et al [6] shows that the resonance frequency for Luminity is higher than 1.5 MHz. New measurements should be performed using higher frequencies.

The results for the different PM applications show that a positive phased signal, using twice the last half amplitude pulse minus the full amplitude pulse, and ultra- or second harmonic imaging gives the highest increase of power fraction for increasing Luminity concentrations. For further measurements it is recommended to use two period pulses for power and phase modulation measurements, this to reduce the amplitude difference between the two phased signals due to starting artefacts from the transducer. If this does not suffice amplitude correction has to be implemented for future measurements.

Several measurements showed a change in response for measurements performed later during the experiment. For further measurements it is recommended to use the dilutions for fewer measurements to see if this improves the results. Also more research on the bubble stability may help explain this.

The results, shown in appendix H, for the backscatter by tissue show the negative effect of nonlinear distortion on imaging. The CTR, which is an important measure for the performance of a contrast detection modes, is reduced with a maximum of 60%. The unexpected high backscatter from tissue for low Luminity concentrations and harmonic imaging makes the results from this measurement questionable. A new measurement using harmonic imaging is recommended to show if this measurement was disturbed.

A possibility to compensate for the CTR reduction due to the backscatter by tissue is the use of nonlinearity measurements. Due to the large difference in nonlinearity between tissue and UCA, the tissue will only reflect the nonlinear distorted US and not introduce new nonlinear distortion, like UCA does. This could be an interesting topic for further research.
7 Bibliography


<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO</td>
<td>Cardiac Output</td>
</tr>
<tr>
<td>CTR</td>
<td>Contrast to Tissue Ratio</td>
</tr>
<tr>
<td>EF</td>
<td>Ejection Fraction</td>
</tr>
<tr>
<td>FIR</td>
<td>Finite Impulse Response</td>
</tr>
<tr>
<td>FPT</td>
<td>First Passage Time</td>
</tr>
<tr>
<td>IDC</td>
<td>Indicator Dilution Curve</td>
</tr>
<tr>
<td>LDRW</td>
<td>Local Density Random Walk</td>
</tr>
<tr>
<td>LSE</td>
<td>Least Square Estimation</td>
</tr>
<tr>
<td>MI</td>
<td>Mechanical Index</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>MRT</td>
<td>Mean Residence Time</td>
</tr>
<tr>
<td>MTT</td>
<td>Mean Transit Time</td>
</tr>
<tr>
<td>PBV</td>
<td>Pulmonary Blood Volume</td>
</tr>
<tr>
<td>ROI</td>
<td>Region Of Interest</td>
</tr>
<tr>
<td>TEE</td>
<td>Trans-Esophageal Echocardiography</td>
</tr>
<tr>
<td>TTE</td>
<td>Trans-Thoracic Echocardiography</td>
</tr>
<tr>
<td>UCA</td>
<td>Ultrasound Contrast agent</td>
</tr>
<tr>
<td>US</td>
<td>Ultrasound</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>a</td>
<td>Amplitude of the fundamental (send) frequency</td>
</tr>
<tr>
<td>A</td>
<td>Area cross-section</td>
</tr>
<tr>
<td>(\alpha)</td>
<td>Thermal and viscous attenuation coefficient</td>
</tr>
<tr>
<td>b</td>
<td>Amplitude of the second harmonic frequency</td>
</tr>
<tr>
<td>B</td>
<td>Magnetic field strength</td>
</tr>
<tr>
<td>(\beta)</td>
<td>Nonlinearity coefficient</td>
</tr>
<tr>
<td>(C_i)</td>
<td>Concentration indicator</td>
</tr>
<tr>
<td>(C_p)</td>
<td>Specific heat at constant pressure</td>
</tr>
<tr>
<td>(C_v)</td>
<td>Specific heat at constant volume</td>
</tr>
<tr>
<td>(C_{vu})</td>
<td>Concentration indicator in unknown volume</td>
</tr>
<tr>
<td>(c_0)</td>
<td>Unperturbed ultrasound speed in medium</td>
</tr>
<tr>
<td>(\delta)</td>
<td>Diffusivity US</td>
</tr>
<tr>
<td>(\delta_{rad})</td>
<td>Dampening due to re-radiation</td>
</tr>
<tr>
<td>(\delta_{tot})</td>
<td>Total dampening</td>
</tr>
<tr>
<td>(\delta_{th})</td>
<td>Dampening due to thermal conduction</td>
</tr>
<tr>
<td>(\delta_{vis})</td>
<td>Dampening due to the viscosity of the medium</td>
</tr>
<tr>
<td>(\varepsilon)</td>
<td>Acoustic Mach number</td>
</tr>
<tr>
<td>(\Phi)</td>
<td>Flow</td>
</tr>
<tr>
<td>(f)</td>
<td>Frequency</td>
</tr>
<tr>
<td>(f_{r,free})</td>
<td>Resonance frequency free air bubble</td>
</tr>
<tr>
<td>(f_r)</td>
<td>Resonance frequency encapsulated UCA bubble</td>
</tr>
<tr>
<td>(f_R)</td>
<td>Measured frequency at receiver</td>
</tr>
<tr>
<td>(f_T)</td>
<td>Measured frequency at transmitter</td>
</tr>
<tr>
<td>(k)</td>
<td>Wave number</td>
</tr>
<tr>
<td>(K)</td>
<td>Heat conductivity number</td>
</tr>
<tr>
<td>(L)</td>
<td>Distance</td>
</tr>
<tr>
<td>(m_i)</td>
<td>Indicator doses</td>
</tr>
<tr>
<td>(P)</td>
<td>Acoustic pressure</td>
</tr>
<tr>
<td>(P_0)</td>
<td>Unperturbed acoustic pressure</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>$P_{g0}$</td>
<td>Initial gas pressure inside the bubble</td>
</tr>
<tr>
<td>$P_{neg}$</td>
<td>Peak negative acoustic pressure</td>
</tr>
<tr>
<td>$P_v$</td>
<td>Vapour pressure</td>
</tr>
<tr>
<td>$\theta$</td>
<td>Thermal expansion coefficient</td>
</tr>
<tr>
<td>$r^2$</td>
<td>Determination coefficient</td>
</tr>
<tr>
<td>$\sigma_a$</td>
<td>Absorption cross-section</td>
</tr>
<tr>
<td>$\sigma_e$</td>
<td>Extinction cross-section</td>
</tr>
<tr>
<td>$\sigma_s$</td>
<td>Scattering cross-section</td>
</tr>
<tr>
<td>$\sigma_{st}$</td>
<td>Surface tension bubble</td>
</tr>
<tr>
<td>$R$</td>
<td>Bubble radius</td>
</tr>
<tr>
<td>$R_0$</td>
<td>Equilibrium bubble radius</td>
</tr>
<tr>
<td>$\rho$</td>
<td>Density medium</td>
</tr>
<tr>
<td>$\rho_b$</td>
<td>Resistivity blood</td>
</tr>
<tr>
<td>$\rho_0$</td>
<td>Unperturbed medium density</td>
</tr>
<tr>
<td>$\rho_l$</td>
<td>Density liquid</td>
</tr>
<tr>
<td>$\rho_{l0}$</td>
<td>Density unperturbed liquid</td>
</tr>
<tr>
<td>$\rho_g$</td>
<td>Density gas</td>
</tr>
<tr>
<td>$\rho_{g0}$</td>
<td>Density unperturbed gas</td>
</tr>
<tr>
<td>$S$</td>
<td>Entropy medium</td>
</tr>
<tr>
<td>$S_0$</td>
<td>Entropy unperturbed medium</td>
</tr>
<tr>
<td>$\tau$</td>
<td>Retarded time</td>
</tr>
<tr>
<td>$\tau_e$</td>
<td>Ejection time</td>
</tr>
<tr>
<td>$\mu$</td>
<td>Thermal viscosity medium</td>
</tr>
<tr>
<td>$\mu_b$</td>
<td>Bulk viscosity medium</td>
</tr>
<tr>
<td>$v$</td>
<td>Particle velocity</td>
</tr>
<tr>
<td>$v_c$</td>
<td>Particle velocity compressional phase</td>
</tr>
<tr>
<td>$v_r$</td>
<td>Particle velocity rarefractional phase</td>
</tr>
<tr>
<td>$V_B$</td>
<td>Speed of blood</td>
</tr>
<tr>
<td>$V_i$</td>
<td>Volume indicator</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------</td>
</tr>
<tr>
<td>$V_u$</td>
<td>Unknown blood volume</td>
</tr>
<tr>
<td>$\omega$</td>
<td>Angular frequency</td>
</tr>
<tr>
<td>$\chi$</td>
<td>Polytrophic gas index</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>Ratio of specific heats</td>
</tr>
<tr>
<td>$Z$</td>
<td>Impedance</td>
</tr>
<tr>
<td>$Z_t$</td>
<td>Impedance tissue</td>
</tr>
<tr>
<td>$Z_b$</td>
<td>Impedance blood</td>
</tr>
</tbody>
</table>
A. Calibration protocol

A.1. Storage Luminity UCA
- The Luminity (Brystol Myers Squibb) vials are stored at a temperature ranging between 2°C and 8°C.

A.2. Preparation dilutions
- Before activation, the Luminity vial has to warm up till room temperature. To this end the vial is taken out of the cooling unit between 15 and 30 minutes before activation.
- Before use the Luminity is activated by shaking the vial for 45 seconds using a Vialmix (Brystol Myers Squibb). After activation the transparent fluid in the vial turns into a milky white suspension.
- During the activation with the Vialmix the vial with the Luminity heats up. After the activation the vial stands for 15 to 30 minutes to cool down to room temperature [18].
- Before preparing the first dilution the Activated Luminity is hand shaken gently for 10 seconds to reactivate the Luminity.
- The used volumes of saline have to be measured precisely, because a volume error causes an error in the concentration of the dilution of the same size as the volume error. Due to the small concentrations of interest, up to three dilution steps are necessary to reach the smallest concentrations. In these three dilution steps the concentration errors add up.
- 1 ml of saline is withdrawn from the first 100 ml of saline to keep the total volume of the dilution 100 ml after adding the activated Luminity (dilution M1).
- 1 ml Luminity is drawn from the activated vial using a 20 gauge needle and a 1 ml syringe to prevent bubble destruction. The Luminity is drawn from the centre of the inverted vial and no air should be injected into the Luminity vial.
- After preparation the bottle with the Luminity dilution is gently shaken for 10 seconds before further use.
- After the preparation of the first dilution all other dilution can be made from this dilution according to table A.1 using the same steps. The used Pipettes should have a maximum volume close to the amount that has to be drawn to keep the errors small.

Table A.1: The dilution scheme for the Luminity calibration measurements. The dilutions marked with an M are only used for the preparation of smaller dilutions and not for the calibration measurements.

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Luminity</th>
<th>Saline</th>
<th>%</th>
<th>Conc. C₃F₈</th>
<th>C₅F₈</th>
</tr>
</thead>
<tbody>
<tr>
<td>M0</td>
<td>Vial Luminity (1.5 mL)</td>
<td>-</td>
<td>100%</td>
<td>150000 µL/L</td>
<td>225 µL</td>
</tr>
<tr>
<td>M1</td>
<td>1 mL of M0 Added to</td>
<td>99 mL</td>
<td>1%</td>
<td>1500 µL/L</td>
<td>150 µL</td>
</tr>
<tr>
<td>M2</td>
<td>20 mL of M1 Added to</td>
<td>80 mL</td>
<td>0.1%</td>
<td>300 µL/L</td>
<td>30 µL</td>
</tr>
<tr>
<td>M3</td>
<td>10 mL of M2 Added to</td>
<td>90 mL</td>
<td>0.01%</td>
<td>30 µL/L</td>
<td>3 µL</td>
</tr>
<tr>
<td>1</td>
<td>13 mL of M1 Added to</td>
<td>487 mL</td>
<td>0.026%</td>
<td>39 µL/L</td>
<td>19.5 µL</td>
</tr>
<tr>
<td>2</td>
<td>6.5 mL of M1 Added to</td>
<td>493.5 mL</td>
<td>0.013%</td>
<td>19.5 µL/L</td>
<td>9.75 µL</td>
</tr>
<tr>
<td>3</td>
<td>16 mL of M2 Added to</td>
<td>484 mL</td>
<td>0.0064%</td>
<td>9.6 µL/L</td>
<td>4.8 µL</td>
</tr>
<tr>
<td>4</td>
<td>8 mL of M2 Added to</td>
<td>492 mL</td>
<td>0.0032%</td>
<td>4.8 µL/L</td>
<td>2.4 µL</td>
</tr>
<tr>
<td>5</td>
<td>4 mL of M2 Added to</td>
<td>496 mL</td>
<td>0.0016%</td>
<td>2.4 µL/L</td>
<td>1.2 µL</td>
</tr>
<tr>
<td>6</td>
<td>20 mL of M3 Added to</td>
<td>480 mL</td>
<td>0.0008%</td>
<td>1.2 µL/L</td>
<td>0.6 µL</td>
</tr>
<tr>
<td>7</td>
<td>20 mL of M3 Added to</td>
<td>490 mL</td>
<td>0.0004%</td>
<td>0.6 µL/L</td>
<td>0.3 µL</td>
</tr>
<tr>
<td>8</td>
<td>Reference dilution</td>
<td>500 mL</td>
<td>0%</td>
<td>0 µL/L</td>
<td>0 µL</td>
</tr>
</tbody>
</table>

A.3. Calibration set-up
- For the measurement of the acoustic intensity an Omniplane III (Philips Medical systems) TEE US probe is used.
• The Omniplane III TEE probe is placed close to the wall of the beaker glass with the transducer tip facing towards the absorbing material as shown in figure 3.1. The transducer tip is placed in the middle of the Luminity dilution to minimize the backscatter from the bottom of the beaker glass and the surface of the dilution.

• The walls of the beaker glass are covered by acoustic absorbing material, as shown in figure 3.1, to minimize the disturbance due to backscatter from the measurement set-up.

• The 1 L beaker glass with acoustic absorber is filled with 500 mL of Luminity dilution.

• A magnetic stirrer is used to keep the dilutions uniformly mixed during the calibration measurements.

• After each set of calibration measurement with one concentration the measurement set-up is cleaned with water and dried before being filled with a new Luminity dilution.

• All calibration measurements are performed with a low MI of 0.1 and the exposure to the US (US) is kept short (5s). This is done to minimize the destruction of the bubbles.

• The calibrations are performed using two different US scanners, a Sonos 5500 (Philips Medical systems) and an iE33 (Philips Medical Systems).

• The settings for the different imaging modes and US scanners are shown in table A.2 and A.3.

<table>
<thead>
<tr>
<th>Imaging Mode</th>
<th>Gain</th>
<th>Compensation</th>
<th>Post processing</th>
<th>Depth</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCE Harmonic</td>
<td>65</td>
<td>80</td>
<td>A (linear)</td>
<td>4 cm</td>
</tr>
<tr>
<td>TCE Ultra-Harmonic</td>
<td>65</td>
<td>80</td>
<td>A (linear)</td>
<td>4 cm</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Imaging Mode</th>
<th>Gain</th>
<th>Compensation</th>
<th>Post processing</th>
<th>Depth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue Harmonic</td>
<td>50</td>
<td>80</td>
<td>iScann off</td>
<td>4 cm</td>
</tr>
</tbody>
</table>

• The setting of the US machine and the measurement set-up are kept constant for all calibration sets.

A.4. Analyses of the calibration measurements

• The measured intensity from the Sonos 5500 is exported in DSR Tiff. The measured intensity from the iE33 is exported in Dicom. Both are standard formats to register and transport US images.

• The measured acoustic intensities are exported with minimal pre-processing from the US scanner (post processing A / iScann off) and quantified using a region of interest (ROI) and Qlab (Philips Medical systems).

• The ROI for the measurement of the acoustic intensity is placed immediately beyond the near-field zone as shown in figure 3.3 This to avoid artefacts from the near field zone and to minimize the effects of shadowing.

• The analyses of the measured acoustic intensity is done using Matlab (the Mathworks).

• The acoustic intensity for a concentration of Luminity is determined by the mean acoustic intensity measured from the ROI from the five first frames.

• The data fitting is done using the least square error fitting algorithms available in Matlab and the determination coefficient \( r^2 \) is used to express the correlation between the data and the fit.
A.5. Transfer measurement data iE33

1. Setting the IP address by double clicking the network connection icon.

2. Open the properties dialog for the Local Area Connection by double clicking the active network connection icon.

3. Select Internet Protocol (TCP/IP) and click on the properties button.

4. Fill in the IP address as show below and confirm using the OK button.
5. Make sure the firewall is deactivated before copying the measurements from the iE33 to the laptop.
6. Connect the iE33 with the laptop using a normal network cable.
7. The measurements can be transferred to the laptop using the send to network option on the iE33.
8. The data is placed in the directory: C:\Program Files\Philips\QLAB\Images\ 

A.6. Transfer measurement data Sonos 5500

1. Save the measurements on the MO drive
2. Make sure the firewall is deactivated before copying the measurements from the Sonos 5500 to the laptop.
3. Connect the Sonos 5500 with the laptop using a normal network cable.
4. Double-click the batch file icon "z1gs.bat" on the desktop to install the connection between the MO drive on the Sonos 5500 and the laptop.
   - Double click "My computer" to open the i-share connection with the MO disk on the Sonos 5500.
   - Double click "i-share on Sonos 5500" to copy the files from the MO disk to the laptop.
B. Measurement protocol nonlinear distortion

B.1. Storage Luminity UCA

- The Luminity (Bristol Myers Squibb) vials are stored at a temperature ranging between 2°C and 8°C.

B.2. Preparation dilutions

- Before activation the Luminity vial has to warm up till room temperature. To this end the vial is taken out of the cooling unit between 15 and 30 minutes before activation.
- Before use the Luminity is activated by shaking the vial for 45 seconds using a Vialmix (Bristol Myers Squibb). After activation the transparent fluid in the vial turns into a milky white suspension.
- During the activation with the Vialmix the vial with the Luminity heats up. After the activation the vial stands for 15 to 30 minutes to cool down to room temperature [18].
- Before preparing the first dilution the Activated Luminity is hand shaken gently for 10 seconds to reactivate the Luminity.
- The used volumes of saline have to be measured precisely, because a volume error causes an error in the concentration of the dilution of the same size as the volume error. Due to the small concentrations of interest, up to three dilution steps are necessary to reach the smallest concentrations. In these three dilution steps the concentration errors add up.
- 1 ml of saline is withdrawn from the first 100 ml of saline to keep the total volume of the dilution 100 ml after adding the activated Luminity (dilution M1).
- 1 ml Luminity is drawn from the activated vial using a 20 gauge needle and a 1 ml syringe to prevent bubble destruction. The Luminity is drawn from the centre of the inverted vial and no air should be injected into the Luminity vial.
- After preparation the Luminity dilution is gently shaken for 10 seconds before further use.
- After the preparation of the first dilution all other dilution can be made from this dilution according to table B.1 using the same steps. The used syringes should have a maximum volume close to the amount that has to be drawn to keep the errors small.

Table B.1: The dilution scheme for the Luminity calibration measurements. The dilutions marked with an M are only used for the preparation of smaller dilutions and not for the calibration measurements.

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Luminity</th>
<th>Saline</th>
<th>%</th>
<th>Conc. C₃F₈</th>
<th>C₃F₈</th>
</tr>
</thead>
<tbody>
<tr>
<td>M0</td>
<td>Initial Luminity 1.5 ml</td>
<td>100%</td>
<td>150000 µL/L</td>
<td>225 µL</td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>1 ml of M0 Added to 99 ml</td>
<td>1%</td>
<td>150 µL/L</td>
<td>150 µL</td>
<td></td>
</tr>
<tr>
<td>M2</td>
<td>10 ml of M1 Added to 90 ml</td>
<td>0.1%</td>
<td>150 µL/L</td>
<td>15 µL</td>
<td></td>
</tr>
<tr>
<td>M3</td>
<td>10 ml of M2 Added to 90 ml</td>
<td>0.01%</td>
<td>15 µL/L</td>
<td>1.5 µL</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.2 ml of M1 Added to 98.8 ml</td>
<td>0.012%</td>
<td>18 µL/L</td>
<td>1.8 µL</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.9 ml of M1 Added to 99.1 ml</td>
<td>0.009%</td>
<td>13.5 µL/L</td>
<td>1.35 µL</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.7 ml of M1 Added to 99.3 ml</td>
<td>0.007%</td>
<td>10.5 µL/L</td>
<td>1.05 µL</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5 ml of M2 Added to 95 ml</td>
<td>0.005%</td>
<td>7.5 µL/L</td>
<td>0.75 µL</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2.5 ml of M2 Added to 97.5 ml</td>
<td>0.0035%</td>
<td>3.75 µL/L</td>
<td>0.375 µL</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1.5 ml of M2 Added to 98.5 ml</td>
<td>0.0015%</td>
<td>2.25 µL/L</td>
<td>0.225 µL</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1 ml of M2 Added to 99 ml</td>
<td>0.001%</td>
<td>1.5 µL/L</td>
<td>0.15 µL</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.7 ml of M2 Added to 99.3 ml</td>
<td>0.0007%</td>
<td>1.05 µL/L</td>
<td>0.105 µL</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>5 ml of M3 Added to 95 ml</td>
<td>0.0005%</td>
<td>0.75 µL/L</td>
<td>0.075 µL</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>2.5 ml of M3 Added to 97.5 ml</td>
<td>0.00025%</td>
<td>0.375 µL/L</td>
<td>0.034 µL</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Reference dilution</td>
<td>500 ml</td>
<td>0%</td>
<td>0 µL/L</td>
<td>0 µL</td>
</tr>
</tbody>
</table>
After preparation, all dilutions are gently filled into latex bags to prevent adding extra air bubbles to the dilutions. Latex bags are used because they have a small acoustic backscatter and make it easy to change the different dilutions in the measurement set-up.

The latex bags are sealed using clamps to minimize pressure variations in the bags.

**B.3. Set-up nonlinear propagation measurement**

- For the measurement a single element US transducer (Olympus A302S-SU immersion transducer) is used as transmitter. The used US transducer has a central frequency of 1 MHz and is used to transmit pulse with a frequency of 1, 1.5, and 2 MHz. The focus of the transducer is at 4.3 cm.
- A hydrophone combination (Onda HGL-0400, bandwidth 250 KHz -15 MHz) is be used as receiver to detect the US influenced by the Luminity UCA dilution.
- The signal from the Hydrophone is amplified (Onda AH2010-025) before
- For each frequency three different pulse sequences are transmitted. First an power modulation (PM) sequence of three pulses with amplitudes \(\frac{1}{2}, 1 \) and \(\frac{3}{2}\) with 13 \(\mu\)s between the pulses. Second an PM sequence with the same amplitudes as the first, now with 180° phase difference. Both pulses are shown in figure B.1 and are used to analyze the influence of Luminity on PM and phase shift analyses methods. The third sequence, a sine wave of ten periods, is used to analyze the nonlinear deformation of the US due to Luminity. A long sequence of ten pulses is used because of the larger frequency resolution.

![Figure B.1: Both PM sequences with amplitudes of \(\frac{1}{2}, 1 \) and \(\frac{3}{2}\) and a phase difference of 180°.](image)

- The maximum mechanical index (MI) of all sequences is set to 0.1, which is defined as
  \[
  MI = \frac{P_{neg}}{\sqrt{f}}. \tag{Eq. B.1}
  \]
  Here \(P_{neg}\) is the peak negative pressure and \(f\) the transmitted frequency.

- To determine the MI for all frequencies and pulse sequences a calibration measurement is performed at the focus of the transducer.
- The relation between the measured output voltage of the hydrophone / amplifier and the pressure \((M_L(f))\) is defined as
  \[
  M_L(f) \approx G(f)M_C(f)\frac{C_H}{C_H + C_A} \quad \tag{Eq. B.2}
  \]
  With \(M_C(f)\) being the relation between the output voltage of the hydrophone and the pressure, \(G(f)\) being the gain of the amplifier, \(C_a\) and \(C_a\) being the capacitance of respectively the hydrophone and the amplifier.
• The used setting and desired calibration results are shown in table B.2 for a MI of 0.1.

Table B.2: Settings for a MI of 0.1.

<table>
<thead>
<tr>
<th></th>
<th>1 MHz</th>
<th>1.5 MHz</th>
<th>2 MHz</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{neg}$ (kPa)</td>
<td>100</td>
<td>120</td>
<td>140</td>
</tr>
<tr>
<td>$G(f)$ (dB)</td>
<td>19.88</td>
<td>19.90</td>
<td>19.91</td>
</tr>
<tr>
<td>$G(f)$ (dB)</td>
<td>9.86</td>
<td>9.89</td>
<td>9.90</td>
</tr>
<tr>
<td>$M_c(f)$ (V/Pa)</td>
<td>$2.091\times10^{-7}$</td>
<td>$1.845\times10^{-7}$</td>
<td>$1.692\times10^{-7}$</td>
</tr>
<tr>
<td>$C_h$ (pF)</td>
<td>12.312</td>
<td>12.199</td>
<td>12.093</td>
</tr>
<tr>
<td>$C_a$ (pF)</td>
<td>6.15</td>
<td>6.15</td>
<td>6.16</td>
</tr>
<tr>
<td>$M_l(f)$ (V/Pa)</td>
<td>$1.37\times10^{-6}$</td>
<td>$1.21\times10^{-6}$</td>
<td>$1.11\times10^{-6}$</td>
</tr>
<tr>
<td>Desired output hydrophone (mV)</td>
<td>137</td>
<td>145.2</td>
<td>155.4</td>
</tr>
<tr>
<td>Periods between PM pulses</td>
<td>13</td>
<td>20</td>
<td>26</td>
</tr>
<tr>
<td>Frequency total PM sequence (kHz)</td>
<td>34482.76</td>
<td>35714.29</td>
<td>36363.64</td>
</tr>
<tr>
<td>Amplitude10 period sin wave (mV)</td>
<td>160</td>
<td>250</td>
<td>800</td>
</tr>
<tr>
<td>Amplitude PM, positive start (mV)</td>
<td>260</td>
<td>300</td>
<td>320</td>
</tr>
<tr>
<td>Amplitude PM, negative start (mV)</td>
<td>175</td>
<td>200</td>
<td>220</td>
</tr>
</tbody>
</table>

• The pulse sequences are made using a pulse generator (Agilent, 33220A) and amplified using a RF amplifier (ENI, 240L).
• The output of the hydrophone / amplifier is digitized (NI PCI 5112) to allow further analyses on a pc.
• The sampling rate of the hydrophone signal will be at least 4 times the highest frequency of interest to minimize aliasing. This results in a sampling frequency of at least 40 MHz (Frequencies of interest till fifth harmonic). In practice, we will sample at the maximum of the board, which is 100 MHz.
• The measurement set-up as shown in figure 4.5 is filled with water to match the acoustic impedance of the saline used for the Luminity dilutions so that the backscatter due to different acoustic impedance is minimized.
• The backscatter from the walls of the measurement set-up is minimized by surrounding the latex bags filled with Luminity by acoustic absorbing material (sponges).
• To minimize the measurement error due to air bubbles in water, the measurement set-up is filled one day in advance. By doing this, air bubbles trapped in water while filling the measurement set-up have time to diffuse out. A further reduction of the air bubbles can be achieved by using ice water, which reduces the solvability of air and therefore the amount of air in water that can contribute to build air bubbles.
• All measurements are performed with a low MI of 0.1. This to minimize the destruction of the bubbles, but keep the nonlinear behaviour of the UCA.
• Before use, the latex bag with the Luminity dilution is hand shaken gently for 10 seconds, to insure a uniform mixing of the Luminity UCA.

B.4. Analysis nonlinear propagation measurements
• The measured acoustic pressure is analyzed using Matlab (The Mathworks)
• First the measured acoustic pressure is filtered using a low pass FIR filter with a cute-off frequency of 15 times the send frequency (to ensure a flat pass for frequencies till the forth harmonic) and a filter length of 50 samples.
• The signal of interest is filtered out of the measured pressure signal using a Hamming window.
• The length of the US signal is increased till 4000 samples by adding zero to the end of the signal. This is done to increase the freq. resolution of the DFT and to compare the analysis of different measurements.
• The frequency response of the US signal is determined using the Matlab DFT algorithm.
• The bandwidth from the different harmonic frequency bands is determined by the cute-off frequency. In case the cute-off frequency is wider than 0.1 MHz, the frequency band is limited to 0.1 MHz round the centre frequency.
### C. Discarded measurements

#### Table C.1: Ten period sinusoidal wave: 1 MHz.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Reason for discarding the measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 μL/L</td>
<td>Large amplitude deviation.</td>
</tr>
<tr>
<td>0.75 μL/L</td>
<td></td>
</tr>
<tr>
<td>1.5 μL/L</td>
<td></td>
</tr>
<tr>
<td>2.25 μL/L</td>
<td></td>
</tr>
<tr>
<td>5.25 μL/L</td>
<td></td>
</tr>
<tr>
<td>7.5 μL/L</td>
<td></td>
</tr>
<tr>
<td>13.5 μL/L</td>
<td></td>
</tr>
<tr>
<td>18 μL/L</td>
<td></td>
</tr>
</tbody>
</table>

#### Table C.2: PM wave positive phase: 1 MHz.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Reason for discarding the measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 μL/L</td>
<td>Large amplitude deviation.</td>
</tr>
<tr>
<td>0.375 μL/L</td>
<td>Signal is clipped.</td>
</tr>
<tr>
<td>0.75 μL/L</td>
<td>Large deviation power spectrum.</td>
</tr>
<tr>
<td>1.05 μL/L</td>
<td>2 - PM 1</td>
</tr>
<tr>
<td>1.5 μL/L</td>
<td>2,3</td>
</tr>
<tr>
<td>2.25 μL/L</td>
<td>Signal is clipped.</td>
</tr>
<tr>
<td>5.25 μL/L</td>
<td>Large deviation power spectrum.</td>
</tr>
<tr>
<td>7.5 μL/L</td>
<td>Large amplitude deviation.</td>
</tr>
</tbody>
</table>

#### Table C.3: PM wave negative phase: 1 MHz.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Reason for discarding the measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 μL/L</td>
<td>1 - PM 1</td>
</tr>
<tr>
<td>0 μL/L</td>
<td>2 - PM 2</td>
</tr>
<tr>
<td>0.375 μL/L</td>
<td>Large amplitude deviation.</td>
</tr>
<tr>
<td>0.75 μL/L</td>
<td></td>
</tr>
<tr>
<td>1.5 μL/L</td>
<td>1 - PM 2</td>
</tr>
<tr>
<td>3.75 μL/L</td>
<td>3 - PM 2</td>
</tr>
<tr>
<td>5.25 μL/L</td>
<td>Large amplitude deviation.</td>
</tr>
<tr>
<td>7.5 μL/L</td>
<td></td>
</tr>
<tr>
<td>10.5 μL/L</td>
<td>3 - PM 2</td>
</tr>
<tr>
<td>13.5 μL/L</td>
<td>Large amplitude deviation.</td>
</tr>
<tr>
<td>18 μL/L</td>
<td></td>
</tr>
</tbody>
</table>

#### Table C.4: Ten period sinusoidal wave: 1.5 MHz.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Reason for discarding the measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 μL/L</td>
<td>Large amplitude deviation.</td>
</tr>
<tr>
<td>0.375 μL/L</td>
<td></td>
</tr>
<tr>
<td>0.75 μL/L</td>
<td></td>
</tr>
<tr>
<td>1.5 μL/L</td>
<td></td>
</tr>
<tr>
<td>2.25 μL/L</td>
<td></td>
</tr>
<tr>
<td>7.5 μL/L</td>
<td></td>
</tr>
<tr>
<td>13.5 μL/L</td>
<td></td>
</tr>
</tbody>
</table>
Table C.5: PM wave positive phase: 1.5 MHz.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Reason for discarding the measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 µL/L</td>
<td>2 - PM 2 Large deviation power spectrum.</td>
</tr>
<tr>
<td>0.375 µL/L</td>
<td>1 - PM 2 Large amplitude deviation.</td>
</tr>
<tr>
<td>0.375 µL/L</td>
<td>3 - PM 1 Large amplitude deviation.</td>
</tr>
<tr>
<td>0.75 µL/L</td>
<td>1 Large deviation power spectrum.</td>
</tr>
<tr>
<td>1.05 µL/L</td>
<td>2 Large amplitude deviation.</td>
</tr>
<tr>
<td>1.5 µL/L</td>
<td>1,2,3 Signal is clipped.</td>
</tr>
<tr>
<td>3.75 µL/L</td>
<td>3 Large amplitude deviation.</td>
</tr>
<tr>
<td>5.25 µL/L</td>
<td>1 - PM 2 Large deviation power spectrum.</td>
</tr>
<tr>
<td>5.25 µL/L</td>
<td>3 - PM 3 Large amplitude deviation.</td>
</tr>
<tr>
<td>7.5 µL/L</td>
<td>2 - PM 1,2 Large amplitude deviation.</td>
</tr>
<tr>
<td>10.5 µL/L</td>
<td>1 Large amplitude deviation.</td>
</tr>
<tr>
<td>13.5 µL/L</td>
<td>1 Large amplitude deviation.</td>
</tr>
<tr>
<td>18 µL/L</td>
<td>1 Large amplitude deviation.</td>
</tr>
</tbody>
</table>

Table C.6: PM wave negative phase: 1.5 MHz.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Reason for discarding the measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.375 µL/L</td>
<td>2 - PM 1,2 Large deviation power spectrum.</td>
</tr>
<tr>
<td>1.05 µL/L</td>
<td>1 - PM 1,2 Large deviation power spectrum.</td>
</tr>
<tr>
<td>1.5 µL/L</td>
<td>1 - PM 2 Large deviation power spectrum.</td>
</tr>
<tr>
<td>1.5 µL/L</td>
<td>1 - PM 1 Large amplitude deviation.</td>
</tr>
<tr>
<td>2.25 µL/L</td>
<td>1 - PM 2 Large deviation power spectrum.</td>
</tr>
<tr>
<td>2.25 µL/L</td>
<td>3 - PM 1 Large amplitude deviation.</td>
</tr>
<tr>
<td>3.75 µL/L</td>
<td>2 - PM 1 Large deviation power spectrum.</td>
</tr>
<tr>
<td>3.75 µL/L</td>
<td>3 - PM 2 Large amplitude deviation.</td>
</tr>
<tr>
<td>5.25 µL/L</td>
<td>3 Large amplitude deviation.</td>
</tr>
<tr>
<td>7.5 µL/L</td>
<td>3 Large deviation power spectrum.</td>
</tr>
<tr>
<td>10.5 µL/L</td>
<td>2 - PM 1 Large deviation power spectrum.</td>
</tr>
<tr>
<td>10.5 µL/L</td>
<td>3 - PM 2 Large amplitude deviation.</td>
</tr>
<tr>
<td>13.5 µL/L</td>
<td>1 Large amplitude deviation.</td>
</tr>
<tr>
<td>18 µL/L</td>
<td>1 Large amplitude deviation.</td>
</tr>
</tbody>
</table>
## D. SNRs

Table D.1: SNR for the different frequency bands: 1 MHz.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>46.36</td>
<td>6.01E+05</td>
<td>9.47</td>
<td>43.80</td>
<td>4.80</td>
<td>5.14</td>
<td>0.44</td>
</tr>
<tr>
<td>0.375</td>
<td>118.97</td>
<td>5.85E+05</td>
<td>26.25</td>
<td>36.59</td>
<td>1.95</td>
<td>6.00</td>
<td>11.37</td>
</tr>
<tr>
<td>0.75</td>
<td>52.02</td>
<td>4.54E+05</td>
<td>10.50</td>
<td>22.23</td>
<td>2.77</td>
<td>45.52</td>
<td>3.09</td>
</tr>
<tr>
<td>1.05</td>
<td>271.40</td>
<td>5.06E+06</td>
<td>105.75</td>
<td>197.176</td>
<td>12.90</td>
<td>126.09</td>
<td>30.93</td>
</tr>
<tr>
<td>1.5</td>
<td>297.92</td>
<td>2.26E+06</td>
<td>25.05</td>
<td>957.91</td>
<td>7.30</td>
<td>32.03</td>
<td>6.38</td>
</tr>
<tr>
<td>2.25</td>
<td>59.52</td>
<td>2.63E+06</td>
<td>10.50</td>
<td>5367.95</td>
<td>2.13</td>
<td>53.77</td>
<td>22.26</td>
</tr>
<tr>
<td>3.75</td>
<td>36.05</td>
<td>6.60E+05</td>
<td>8.46</td>
<td>2012.27</td>
<td>4.13</td>
<td>93.81</td>
<td>68.66</td>
</tr>
<tr>
<td>5.25</td>
<td>270.60</td>
<td>9.67E+05</td>
<td>16.08</td>
<td>4679.24</td>
<td>7.86</td>
<td>96.01</td>
<td>193.69</td>
</tr>
<tr>
<td>7.5</td>
<td>153.14</td>
<td>4.88E+06</td>
<td>29.80</td>
<td>182160.05</td>
<td>29.37</td>
<td>219.15</td>
<td>586.90</td>
</tr>
<tr>
<td>10.5</td>
<td>241.41</td>
<td>2.92E+06</td>
<td>91.02</td>
<td>183584.44</td>
<td>25.52</td>
<td>248.19</td>
<td>1818.56</td>
</tr>
<tr>
<td>13.5</td>
<td>329.39</td>
<td>3.00E+06</td>
<td>20.83</td>
<td>147905.97</td>
<td>18.49</td>
<td>406.34</td>
<td>474.92</td>
</tr>
<tr>
<td>18</td>
<td>131.24</td>
<td>1.11E+06</td>
<td>22.49</td>
<td>93073.84</td>
<td>16.41</td>
<td>4684.75</td>
<td>2405.50</td>
</tr>
</tbody>
</table>

Table D.2: SNR for the different frequency bands: 1.5 MHz.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>121.12</td>
<td>6.09E+05</td>
<td>8.04</td>
<td>251.19</td>
<td>1.40</td>
<td>7.97</td>
<td>1.48</td>
</tr>
<tr>
<td>0.375</td>
<td>59.27</td>
<td>7.95E+05</td>
<td>11.65</td>
<td>89.10</td>
<td>1.82</td>
<td>19.16</td>
<td>2.46</td>
</tr>
<tr>
<td>0.75</td>
<td>48.91</td>
<td>2.53E+05</td>
<td>4.09</td>
<td>121.72</td>
<td>1.28</td>
<td>41.00</td>
<td>2.65</td>
</tr>
<tr>
<td>1.05</td>
<td>59.32</td>
<td>9.41E+05</td>
<td>20.38</td>
<td>319.32</td>
<td>3.05</td>
<td>9.84</td>
<td>15.09</td>
</tr>
<tr>
<td>1.5</td>
<td>39.66</td>
<td>4.33E+05</td>
<td>35.23</td>
<td>44.46</td>
<td>6.62</td>
<td>14.29</td>
<td>11.98</td>
</tr>
<tr>
<td>2.25</td>
<td>48.47</td>
<td>1.59E+05</td>
<td>14.90</td>
<td>191.86</td>
<td>0.64</td>
<td>20.18</td>
<td>2.96</td>
</tr>
<tr>
<td>3.75</td>
<td>78.61</td>
<td>7.83E+05</td>
<td>3.35</td>
<td>993.20</td>
<td>5.67</td>
<td>72.08</td>
<td>4.07</td>
</tr>
<tr>
<td>5.25</td>
<td>31.93</td>
<td>2.61E+05</td>
<td>2.69</td>
<td>1487.14</td>
<td>1.66</td>
<td>166.72</td>
<td>21.15</td>
</tr>
<tr>
<td>7.5</td>
<td>21.99</td>
<td>2.53E+05</td>
<td>1.00</td>
<td>2727.89</td>
<td>3.45</td>
<td>2.95</td>
<td>7.08</td>
</tr>
<tr>
<td>10.5</td>
<td>327.25</td>
<td>2.36E+06</td>
<td>13.81</td>
<td>64153.98</td>
<td>11.01</td>
<td>514.09</td>
<td>194.12</td>
</tr>
<tr>
<td>13.5</td>
<td>199.88</td>
<td>1.61E+06</td>
<td>10.98</td>
<td>47630.11</td>
<td>28.68</td>
<td>1827.35</td>
<td>669.12</td>
</tr>
<tr>
<td>18</td>
<td>353.68</td>
<td>1.05E+06</td>
<td>7.74</td>
<td>137166.67</td>
<td>10.47</td>
<td>3564.82</td>
<td>679.12</td>
</tr>
</tbody>
</table>
E. Power fractions

Figure E.1: Sub-harmonic power fraction of total power: 1 MHz.

Figure E.2: Fundamental power fraction of total power: 1 MHz.

Figure E.3: Ultra-harmonic power fraction of total power: 1 MHz.
Figure E.4: Second-harmonic power fraction of total power: 1 MHz.

Figure E.5: Super-harmonic power fraction of total power: 1 MHz.

Figure E.6: Third-harmonic power fraction of total power: 1 MHz.
Figure E.7: Forth-harmonic power fraction of total power: 1 MHz.

Figure E.8: Sub-harmonic power fraction of total power: 1.5 MHz.

Figure E.9: Fundamental power fraction of total power: 1.5 MHz.
Figure E.10: Ultra-harmonic power fraction of total power: 1.5 MHz.

Figure E.11: Second-harmonic power fraction of total power: 1.5 MHz.

Figure E.12: Super-harmonic power fraction of total power: 1.5 MHz.
Figure E.13: Third-harmonic power fraction of total power: 1.5 MHz.

Figure E.14: Fourth-harmonic power fraction of total power: 1.5 MHz.
F. Model initialisation

Figure F.1: Initialization attenuation simple model: 1 MHz.

Figure F.2: Initialization nonlinearity simple mode: 1 MHz.

Figure F.3: Initialization fundamental amplitude: Burger's model, 1 MHz.
Figure F.4: Initialization second-harmonic amplitude: Burger's model, 1 MHz.

Figure F.5: Initialization attenuation simple model: 1.5 MHz.

Figure F.6: Initialization nonlinearity simple model: 1.5 MHz.
Figure F.7: Initialization fundamental amplitude: Burger's model, 1.5 MHz.

Figure F.8: Initialization second-harmonic amplitude: Burger's model, 1.5 MHz.
G. Estimated attenuation and nonlinearity

Figure G.1: Attenuation coefficient found using the simple model: 1 MHz.

Figure G.2: Nonlinearity coefficient found using the simple model: 1 MHz.

Figure G.3: Attenuation coefficient found using Burger's models for $\Gamma < 1$ and $\Gamma >> 1$: 1 MHz.
Figure G.4: Nonlinearity coefficient found using Burger's models for $r < 1$ and $r >> 1$: 1 MHz.

Figure G.5: Attenuation coefficient found using the simple model: 1.5 MHz.

Figure G.6: Nonlinearity coefficient found using the simple model: 1.5 MHz.
Figure G.7: Attenuation coefficient found using Burger’s model for $\Gamma < 1$ and $\Gamma \gg 1$: 1.5 MHz.

Figure G.8: Nonlinearity coefficient found using Burger’s models for $\Gamma < 1$ and $\Gamma \gg 1$: 1.5 MHz.
H. Backscatter from tissue phantom

Figure H.1: Backscatter of nonlinear US by tissue phantom: Ultra-harmonic imaging.

Figure H.2: Backscatter of nonlinear US by tissue phantom: Harmonic imaging.

Figure H.3: Backscatter of nonlinear US by tissue phantom: Power modulation.
I. Power fraction for PM

**Figure 1.1:** Power fraction for PM: Sub harmonic, 1 MHz.

**Figure 1.2:** Power fraction for PM: Fundamental, 1 MHz.

**Figure 1.3:** Power fraction for PM: Ultra harmonic, 1 MHz.
Figure 1.4: Power fraction for PM: Second harmonic, 1 MHz.

Figure 1.5: Power fraction for PM: Super harmonic, 1 MHz.

Figure 1.6: Power fraction for PM: Third harmonic, 1 MHz.
Power Fraction Total Power, Forth harmonic

Figure 1.7: Power fraction for PM: Forth harmonic, 1 MHz.

Power Fraction Total Power, Sub harmonic

Figure 1.8: Power fraction for PM: Sub harmonic, 1.5 MHz.

Power Fraction Total Power, Fundamental

Figure 1.9: Power fraction for PM: Fundamental, 1.5 MHz.
Figure 1.10: Power fraction for PM: Ultra harmonic, 1.5 MHz.

Figure 1.11: CTR for PM: Second harmonic, 1.5 MHz.

Figure 1.12: Power fraction for PM: Super harmonic, 1.5 MHz.
**Figure 1.13**: Power fraction for PM: Third harmonic, 1.5 MHz.

**Figure 1.14**: Power fraction for PM: Forth harmonic, 1.5 MHz.