Subharmonic Imaging of Polymer-Shelled Contrast Agents

Subharmonisk Avbildning av Polymera Kontrastmedel

RÚNAR SIGMUNDSSON
Abstract

The harmonic generation due to the nonlinear behavior of Ultrasound Contrast Agents (UCAs) must be exploited for improved efficiency when imaging vascular targets in the neighborhood of highly echogenic tissue. One may even further improve the efficiency by focusing on the subharmonic generation of the UCAs, which is an even more exclusive property than the generation of higher harmonics, for improved Contrast-to-Tissue ratio (CTR). The aim of this work was first, the design of a set-up for nonlinear imaging of Poly-Vinyl Alcohol (PVA) based UCAs on The Verasonics Research System with special focus on nondestructive Sub-harmonic Imaging. The second part of the work addressed the evaluation of the subharmonic response provided by the agents in the developed setup. Six different imaging techniques were developed. These were Fundamental B-mode imaging (FB), Pulse Inversion imaging (PI), and a Contrast Pulse Sequence based on three pulses (CPS3), with and without a focus on the subharmonic component by the implementation of a Linear Bandpass Filter (LBF). Experiments were performed on a tissue mimicking flow phantom and the performance of the agents for each technique was determined in terms of CTR and CNR. The PVA agents provided a backscattering enhancement of the order of 23 dB through FB imaging. However, the performance of the FB technique was unsatisfactory in terms of CTR. The CPS3 sequence performed best of the six techniques with an improvement of 14 dB and 13 dB in CTR and CNR, respectively, compared with the FB technique. Combining the LBF around the subharmonic component with the multi-pulse techniques of PI and CPS3 resulted in a degraded CTR performance due to significant amount of signals from tissue around the subharmonic component and insufficient subharmonic detection from the PVA agents.
I would like to express my appreciation to the members of the CACTUS Project; Ph.D. student Chen Hongjian and MSc. students Dimitris Evangelou and Mohammed Rashid for their contributions to this work. I would also like to offer my special gratitude to my supervisor Dmitry Grishenkov, Associate Professor at the Department of Medical Engineering at KTH Royal Institute of Technology, for his valuable support during the scope of this project.

Rúnar Sigmundsson
Stockholm, Sweden
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## List of Abbreviations

<table>
<thead>
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<th>Description</th>
</tr>
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<tbody>
<tr>
<td>UCAs</td>
<td>Ultrasound Contrast Agents</td>
</tr>
<tr>
<td>MBs</td>
<td>Microbubbles</td>
</tr>
<tr>
<td>HI</td>
<td>Harmonic Imaging</td>
</tr>
<tr>
<td>SHI</td>
<td>Subharmonic Imaging</td>
</tr>
<tr>
<td>CTR</td>
<td>Contrast-to-Tissue Ratio</td>
</tr>
<tr>
<td>CNR</td>
<td>Contrast-to-Noise Ratio</td>
</tr>
<tr>
<td>PVA</td>
<td>Poly-Vinyl Alcohol</td>
</tr>
<tr>
<td>GUI</td>
<td>Graphical User Interface</td>
</tr>
<tr>
<td>ROI</td>
<td>Region Of Interest</td>
</tr>
<tr>
<td>PI</td>
<td>Pulse Inversion</td>
</tr>
<tr>
<td>AM</td>
<td>Amplitude Modulation</td>
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<tr>
<td>LBF</td>
<td>Linear Bandpass Filter</td>
</tr>
<tr>
<td>CPS</td>
<td>Contrast Pulse Sequence</td>
</tr>
<tr>
<td>CPS3</td>
<td>Contrast Pulse Sequence based on 3 pulses</td>
</tr>
<tr>
<td>FB</td>
<td>Fundamental B-mode</td>
</tr>
<tr>
<td>FB/LBF</td>
<td>Fundamental B-mode combined with a Linear Bandpass Filter</td>
</tr>
<tr>
<td>PI/LBF</td>
<td>Pulse Inversion combined with a Linear Bandpass Filter</td>
</tr>
<tr>
<td>CPS3/LBF</td>
<td>Contrast Pulse Sequence combined with a Linear Bandpass Filter</td>
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Chapter 1

Introduction

1.1 Background

The detection of blood and blood flow in the neighborhood of highly echogenic tissue through conventional B-mode ultrasound imaging is known to be highly insufficient. The weak echoes produced by the red blood cells get buried in the strong echoes from surrounding tissue. This limits the detection of important vascular components, such as the supplying vessels of the myocardium [1].

The introduction of commercially available Ultrasound Contrast Agents (UCAs) in 1991 brought about new possibilities in vascular imaging. Modern UCAs consist of gas-filled microbubbles (MBs), with diameters of 1-10 µm, encapsulated by a 10-200 nm thick shell composed of proteins, lipids or polymers [2–4]. Injected into the blood pool, the fundamental role of UCAs for diagnostic purposes is to enhance the ultrasound backscattering produced by the blood pool as a whole [5, 6]. This backscattering enhancement is provided through two fundamental mechanisms; (1) the immense acoustic impedance mismatch introduced between the agents and the blood pool, and (2) volume pulsations of the MBs due to the constant switching of positive and negative pressure regions within the field of ultrasound. That is, the MBs will contract in the positive pressure region and expand in the negative pressure region. This results in the MBs becoming a source of ultrasound themselves, which radiates radially from their location (Sections A.2, A.3). These periodic volume changes suggest, just as vibrations in other structures, that these oscillations carry a resonance frequency. Insonified at their resonance frequency, the MBs will scatter and absorb ultrasound with a particularly high efficiency [1].

When UCAs were first introduced, the general belief was that the increase in backscattering performance would be sufficient for acceptable vascular detection through conventional ultrasound techniques. The target of interest (e.g. small vessels) should exhibit enough enhancement so that its grayscale level would provide sufficient contrast against the strongly reflecting neighboring tissues. However, this was and is not the case. The backscatter enhancement provided by the UCAs still leaves some vascular structures well below the strong echoes from surrounding tissue. A new approach was needed; a bubble-specific imaging technique [7].
The acoustic behavior of UCAs greatly differs from tissue in their ability to produce harmonics. When the MBs experience sufficiently high acoustic intensity in the field of ultrasound, the volume pulsations of the MBs can be induced to nonlinear oscillations [1]. That is, the volumetric contractions and expansions are not equal. This results in not only backscatter at the fundamental frequency \( f_0 \), but also at higher harmonics \( 2f_0, 3f_0, 4f_0, \text{ etc.} \). The fact that tissue produces low amount of harmonics compared with UCAs promotes the possibility of separating the backscattered echoes produced by the UCAs from those produced by the tissue. The harmonics can be somewhat considered as the signature of the MBs [8]. This is the principle of Harmonic Imaging (HI) (Sections A.4, A.4.2).

An even more exclusive and a more recently exploited acoustic property of the MBs is their ability to generate subharmonics. Again, at sufficient acoustic intensity, the MBs are able to generate signals at half the transmitted frequency \( (f_0/2) \) [9]. This is the foundation of Subharmonic Imaging (SHI) (Section A.5). The fundamental benefit of SHI over HI results in the improved tissue separation. Tissue produces significantly less amount of subharmonics compared with higher harmonics which results in a more efficient tissue suppression and an improved visualization of the UCAs in the vascular system. These findings suggest an improved Contrast-to-Tissue Ratio (CTR) compared with HI [10].

SHI is a relatively new field within the era of Contrast-Enhanced Ultrasound (CEUS) but previous studies have reported the potential of SHI as the superior method over HI for multiple applications such as perfusion estimation and blood vessel detection [10]. However, in a one-probe system, the conventional method of implementing a bandpass filter around the subharmonic component suffers from spectral leakage which must be limited. Spectral leakage of the transmitted signal’s energy into the subharmonic component is the fundamental factor causing image degradation in SHI. Nonlinear imaging techniques, such as Pulse Inversion (PI), Amplitude Modulation (AM) and other Contrast Pulse Sequences (CPS) have proved to be efficient tools in minimizing spectral leakage and to further improve the CTR in SHI (Section A.4.2) [11].

This work aimed at implementing state-of-the-art ultrasound techniques on the Verasonics Research System® for nonlinear imaging of Poly-Vinyl Alcohol (PVA) shelled contrast agents. The PVA agents have multiple advantages over commercially available UCAs for future diagnostic and therapeutic applications, such as high stability, far greater shelf life, and they offer the possibility for multi-modal functionality. Special focus was placed on nondestructive Subharmonic Imaging and the subharmonic response provided by the PVA agents in the developed set-up. To the author’s best knowledge, the PVA-based agents have to this day not been evaluated through similar set-ups and techniques. Additionally, the developed imaging techniques and set-up on The Verasonics Research System will be made available for future research at the ultrasound lab in the School of Engineering Sciences in Chemistry, Biotechnology and Health at KTH Royal Institute of Technology.

1.2 The CACTUS Project

This work is a part of The CACTUS Project. The CACTUS Project, Contrast Agents for CT and Ultrasound, aims at developing micro/nano-construct that can support multimodal contrast-enhanced imaging such as the combination of Computed Tomography (CT) and Ultrasound Imaging.
Chapter 2

Methods

The following chapter includes a discussion on the design and development of ultrasound pulse techniques through The Verasonics Research System applicable for nonlinear imaging of polymeric contrast agents with a special focus on SHI. Several techniques were implemented and compared in terms of image performance. The contrast agents composed of air-filled microbubbles encapsulated in a Poly-Vinyl Alcohol (PVA) shell. Next sections discuss the experimental setup, data processing, the design and implementation of the pulse techniques, as well as performance measurements.

2.1 Experimental Setup

The main components of the experimental setup are shown in Figure 2.1. The components were as follows: (1) Host Computer, (2) The Verasonics Research System, (3) Ultrasound Transducer, (4) Tissue Mimicking Flow Phantom, (5) Peristaltic Pump, and (6) UCAs Suspension. The function of the system as a whole is briefly discussed below and each component is then described in more details in the following sections.

Figure 2.1: The experimental setup. Photo courtesy of Verasonics, Inc. Kirkland, WA, USA, for (2). Photo courtesy of ATS Laboratories, Inc. Norfolk, VA, USA, for (4).
The host computer carried out the programming design of the ultrasound pulse sequences which were to be sent to The Verasonics Research System. The Verasonics Research System served to implement and transmit the pulse sequences to the ultrasound transducer and receive the backscattered signals detected by the transducer. The received echoes were then sent back to the host computer for post processing. The transducer was placed to transmit vertically and perpendicular downwards onto a flow channel in the tissue mimicking phantom. The UCAs, diluted in degassed water, were pumped from a beaker by the peristaltic pump through the flow channel in the phantom. The solution ended up again in the beaker to complete the flow circuit. A magnetic stirrer was placed under the beaker to ensure sufficient homogeneity of the UCAs suspension.

2.1.1 Host Computer

The ultrasound pulse sequence designs as well as the post processing of the received signals were carried out on the host computer. The programming was designed as Mathworks MATLAB® scripts and all ultrasound parameters and sequences were controlled through a set of MATLAB structures. The MATLAB scripts also specified data processing of the received Radio Frequency (RF) data as well as the image display [12].

It’s worth mentioning that some of the MATLAB scripts were adapted from example scripts provided by Verasonics to provide relevant features for the intended purpose. Additional external functions were programmed for more convenient post-processing and user interface. The MATLAB scripts were programmed in such a way that when running the setup, for a particular ultrasound sequence, and sufficient amount of RF data had been acquired to make up an image, real-time data was presented to the user. These were; (1) RF Data Display, which presented the RF data for a single channel (transducer element), (2) Image Display, which presented the reconstructed RF data scaled to a 256 level grayscale map, and (3) Amplitude Spectrum Display, which computed the amplitude spectrum of the RF data for the specific channel, within a region of interest (ROI) as described by Figure 2.4. At last, (4) A Graphical User Interface (GUI) was presented, which allowed for altering some fundamental parameters on run-time such as output voltage, focus depth, channel selection and time gain compensation.

2.1.2 The Verasonics Research System

The Verasonics Research System included the Verasonics Hardware (Figure 2.1, component (2)) and the Verasonics Software which was essentially installed on the host computer [12]. The Verasonics Research System allowed for a flexible design of transmitting, receiving and processing ultrasound. The system delivered to the user essentially all aspects of a modern ultrasound system and offered the possibility for new designs in ultrasound processing and acquisition [12–14].

2.1.3 Ultrasound Transducer

The Philips ATL L7-4 linear ultrasound transducer was used for transmitting and receiving. The transducer is known to be a suitable choice for vascular and small part applications. The transducer consisted of a linear array, carrying 128 elements and a 38 mm field of view. The -6 dB bandwidth limits of the transducer were at 4 and 7 MHz [15].
2.1.4 Tissue Mimicking Flow Phantom

A Peripheral Vascular Doppler Flow Phantom (Model 524) from ATS Laboratories served as the tissue mimicking medium in the experimental setup (Figure 2.2). The phantom included four circular flow channels with diameters of 2, 4, 6, and 8 mm located 15.0 mm below the scan surface [16]. Experiments were performed on the 6 and 8 mm tube. The UCAs suspension was pumped through the 6 mm flow channel while the 8 mm flow channel contained degassed water. Additional specifications for the Model 524 are presented in Table 2.1.

![Figure 2.2: Phantom Model 524. Photo courtesy of ATS Laboratories, Inc. Norfolk, VA USA.](image)

Table 2.1: General specifications for Phantom Model 524 [16].

<table>
<thead>
<tr>
<th>Tissue Mimicking Material</th>
<th>Urethane rubber</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed of Sound</td>
<td>1450 m/s ± 1.0% at 23°C</td>
</tr>
<tr>
<td>Attenuation Coefficient</td>
<td>0.5 dB/cm/MHz ± 5.0%</td>
</tr>
</tbody>
</table>

2.1.5 Peristaltic Pump

A Watson-Marlow Sci 323 peristaltic pump was used to keep a continuous flow of the UCAs suspension through the flow circuit to ensure sufficient presence of MBs in the scanning area. The flow was kept as low as possible just in order to prevent the ultrasound beam from pushing the MBs out of the field of view. The pump was set at 20 RPM which resulted in an estimated volumetric flow of 44 ml/min in a pulsed manner.

2.1.6 Ultrasound Contrast Agents

The UCAs suspension consisted of air-filled MBs encapsulated in a Poly-Vinyl Alcohol (PVA) shell. The PVA agents were manufactured following the procedure previously described by Paradossi et al. The process for obtaining these MBs involved foaming a modified PVA solution where a cross-linking reaction takes place at the water/air interface. Floating MBs were separated and extensively washed in separatory funnels [17].

The fundamental benefits of the PVA based polymer shells are; (1) ease of fabrication, (2) biocompatibility and safety within the physiological system, (3) shell stability which limits diffusion out of the gas core, providing longer imaging time, and (4) far greater shelf-life, compared with traditional lipidic agents [3, 17]. However, the low elasticity compared with traditional lipidic MBs limits the amount of oscillations, weaker echoes are produced and higher concentrations are needed for acceptable detection [3].

The PVA batch obtained from the fabrication process was diluted in degassed water in order to obtain a final concentration of $10^6$ MBs per milliliter ($10^6$ ml$^{-1}$). A magnetic stirrer ensured homogeneous mixing of the solution during experiments. The resonance frequency of the PVA MBs was estimated to be at 7-10 MHz according to previous studies by Grishenkov et al. [18, 19].
2.2 Data Processing

Before transferring the backscattered echoes, received by the L7-4 transducer, to the host computer, The Verasonics Research System first performed some essential signal processing (Figure 2.3). The received echoes were first amplified as a function of time according to the Time Gain Compensation settings (TGC), set by the user. The signals were then digitized by a 12-bit A/D converter at a sample rate of 4 samples per wave (sample frequency of $4f_0$), which was the maximum sampling rate allowed by the system. The digitized signals then passed through two filters in order to eliminate any DC components and, if needed, to shape the spectrum further. An apodization array then scaled the signals which were to be stored in a local memory before being transferred to the host computer [13]. The received signals were further processed on the host computer as previously discussed in Section 2.1.1.

Figure 2.3: Receiving signals pathway within the Verasonics Hardware. Adapted from [13].


2.3 Performance Measurements

The performance measurements were based on two parameters; Contrast-to-Noise Ratio (CNR), and Contrast-to-Tissue Ratio (CTR). Samples from three ROI were used for the calculations (Figure 2.4). Each ROI composed of 2000 pixels, 50 pixels in width (4 mm) and 40 pixels in height (3.2 mm), at identical depths. It’s worth mentioning that the reconstruction process provided by The Verasonics Research System compressed each value with a square root function before mapping it to a grayscale level. For quantitative analysis on the grayscale images, the compression of the grayscale map was set to 50% and each pixel value squared to obtain the actual quantities produced by the reconstruction [13]. The performance parameters were calculated using Equations 2.1 and 2.2 and averaged over ten consecutively acquired imaging frames.

\[
CNR = 20 \log \left( \frac{P_{PVA}}{P_{WATER}} \right) \tag{2.1}
\]

where \(P_{PVA}\) is the mean of the actual pixel quantity within the ROI of PVA and \(P_{WATER}\) is the mean of the actual pixel quantity within the ROI of water.

\[
CTR = 20 \log \left( \frac{P_{PVA}}{P_{TISSUE}} \right) \tag{2.2}
\]

where \(P_{TISSUE}\) is the mean of the actual pixel quantity within the ROI of the tissue mimicking material.

![Figure 2.4: The ROIs used for performance measurements of the PVA suspension, the tissue mimicking material and the degassed water.](image)

2.4 Ultrasound Pulse Techniques

Different pulse techniques were implemented; (1) Fundamental B-mode (FB), (2) Combined Fundamental B-mode and Linear Bandpass Filtering over the subharmonic component (FB/LBF), (3) PI, (4) Combined PI and LBF (PI/LBF), (5) Contrast Pulse Sequence based on a three pulses (CPS3), and (6) Combined CPS3 and LBF (CPS3/LBF). The CPS3 can be regarded as a 3-pulse version of a combined PI and AM. The techniques are summarized in Table 2.2 as well as in Figure 2.5.
For every pulse technique implemented the following design and parameters were kept constant for all measurements; (1) Each pulse consisted of a center frequency of \( f_0 = 6.92 \) MHz. For the discrete set of transmission frequencies provided by the Verasonics system, the 6.92 MHz was chosen so that the transmission frequency and the subharmonic component (3.46 MHz) were as close to the L7-4 transducer’s bandwidth as possible (4-7 MHz). Also, the 6.92 MHz was an acceptable choice in terms of the MBs resonance frequency. (2) Each pulse consisted of 4 cycles per pulse. Shi et al. reported that the subharmonic component grows with increased number of cycles for short pulses (e.g. <16 cycles). The trade-off is a decrease in spatial resolution when the number of cycles in a pulse is increased [20]. (3) Each scan was focused with a 128 rayline focusing at a focal depth of 60 wavelengths, which was approximately the center of the 6 mm flow channel which contained the PVA suspension.

The peak-to-peak voltage of the transmit waveform (Figure 2.5) was set to 60 V (30 V for +0.5 pulses). This ensured sufficient excitation of the MBs without causing any visible bubble destruction which allowed for continuous imaging of the agents.

The parameters that varied between the different imaging techniques were; (1) number of transmissions per scan, (2) waveform polarity, (3) waveform amplitude, and (4) the implementation of a LBF around the subharmonic component. The techniques are summarized in terms of polarity, amplitude and the implementation of the LBF in Table 2.2. The reader is referred to Sections A.4.1 and A.4.2 as well as Figure A.3 for more detailed information on each imaging technique.

![Figure 2.5: The shape of the actual transmit waveforms for each imaging technique.](image)

<table>
<thead>
<tr>
<th>Technique</th>
<th>Transmission Parameters</th>
<th>LBF Implemented</th>
</tr>
</thead>
<tbody>
<tr>
<td>FB</td>
<td>+1</td>
<td>no</td>
</tr>
<tr>
<td>FB/LBF</td>
<td>+1</td>
<td>yes</td>
</tr>
<tr>
<td>PI</td>
<td>+1, -1</td>
<td>no</td>
</tr>
<tr>
<td>PI/LBF</td>
<td>+1, -1</td>
<td>yes</td>
</tr>
<tr>
<td>CPS3</td>
<td>+0.5, -1, +0.5</td>
<td>no</td>
</tr>
<tr>
<td>CPS3/LBF</td>
<td>+0.5, -1, +0.5</td>
<td>yes</td>
</tr>
</tbody>
</table>

Table 2.2: Summary of transmission parameters for the imaging techniques.
The LBF around the subharmonic component was designed according to requirements set by the Verasonics system. The Verasonics system accepted the filter coefficients for a symmetric 10th-order Finite Impulse Response filter (FIR) [14]. The ideal LBF for this work should enhance the subharmonic component from the UCAs while suppressing tissue signals at other frequencies, especially the fundamental frequency [21]. The main trade-off in the design was between the fractional bandwidth (FB) of the filter and the stopband attenuation (SA) where

\[
FB = \frac{2f_B}{f_c} \times 100\% \quad (2.3)
\]

\[
SA = A_{pass} - A_{stop} \quad (2.4)
\]

and \(f_B\) is one-half of the defined passband, \(f_c\) is the center of the passband and SA is defined in terms of dB between the amplitude of the passband, \(A_{pass}\), and the amplitude of the stopband, \(A_{stop}\) [21]. With the previously defined parameters and the requirements set by the Verasonics system, an LBF with center frequency of 3.46 MHz (\(f_0/2\)), an equiripple behavior and a stopband attenuation of 20 dB was designed, with full stopband attenuation (20 dB) at the fundamental frequency (Figure 2.6).

![Figure 2.6: Magnitude response of the Linear Bandpass Filter.](image_url)
Chapter 3

Results

Figure 3.1 presents the acquired grayscale images for each imaging technique. The grayscale images were averaged over ten consecutively acquired imaging frames for performance measurements. The layout of each grayscale image follows the same principle as was explained in Figure 2.4. The three images in the left column show the results for each of the imaging sequence previously explained by Figure 2.5. The right column shows the same sequences but with the subharmonic specific imaging through the implementation of the LBF previously described in Section 2.4.

(a) Grayscale image with FB imaging.  
(b) Grayscale image with FB/LBF imaging.  
(c) Grayscale image with PI imaging.  
(d) Grayscale image with PI/LBF imaging.  
(e) Grayscale image with CPS3 imaging.  
(f) Grayscale image with CPS3/LBF imaging.

Figure 3.1: Grayscale images acquired with the different imaging techniques previously described.
Figure 3.2 presents the amplitude spectrum of the RF data for each imaging technique acquired from within the three ROI according to the methodology described in Figure 2.4. Each spectrum is the average of the same ten acquired RF data which provided the grayscale images above. For each graph, the red line represents the frequency response within the PVA ROI and similarly is the green line representing the tissue mimicking material and the blue line representing the degassed water ROI. As previously mentioned, the transmitted frequency was at 6.92 MHz and the subharmonic component at 3.46 MHz. Significant changes in tissue response between the FB technique and the multi-pulse techniques of PI and CPS3 can be clearly noticed. Also, the implementation of the subharmonic LBF provides a distinct change in the shape of the amplitude spectra.

(a) Amplitude spectrum with FB for the 3 ROI.  
(b) Amplitude spectrum with FB/LBF for the 3 ROI.  
(c) Amplitude spectrum with PI for the 3 ROI.  
(d) Amplitude spectrum with PI/LBF for the 3 ROI.  
(e) Amplitude spectrum with CPS3 for the 3 ROI.  
(f) Amplitude spectrum with CPS3/LBF for the 3 ROI.

Figure 3.2: Acquired amplitude spectra for each imaging technique.
Figures 3.3a and 3.3b present the performance results in terms of CTR and CNR, respectively. The errorbars represent the standard error ($\sigma_x$, $\sigma_x = \frac{\sigma}{\sqrt{n}}$, where $\sigma$ is the standard deviation of a population and $n = 10$ is the number of samples within a population.

(a) CTR of each imaging technique.  
(b) CNR of each imaging technique.

Figure 3.3: Acquired performance parameters in terms of CTR and CNR.

Table 3.1 summarizes the performance parameters for each imaging technique with standard errors.

<table>
<thead>
<tr>
<th></th>
<th>CTR [dB]</th>
<th>CNR [dB]</th>
</tr>
</thead>
<tbody>
<tr>
<td>FB</td>
<td>5.53 ±0.13</td>
<td>22.98 ±0.13</td>
</tr>
<tr>
<td>FB/LBF</td>
<td>7.03 ±0.17</td>
<td>24.52 ±0.19</td>
</tr>
<tr>
<td>PI</td>
<td>16.08 ±0.20</td>
<td>36.83 ±0.21</td>
</tr>
<tr>
<td>PI/LBF</td>
<td>13.92 ±0.28</td>
<td>30.20 ±0.32</td>
</tr>
<tr>
<td>CPS3</td>
<td>19.52 ±0.63</td>
<td>36.14 ±0.64</td>
</tr>
<tr>
<td>CPS3/LBF</td>
<td>14.63 ±0.46</td>
<td>27.65 ±0.55</td>
</tr>
</tbody>
</table>

As can be seen in Figure 3.3 and Table 3.1, the PI and CPS3 provide significant improvements in terms of both CTR and CNR. However, the subharmonic specific LBF implementation results in a slight degradation in image performance compared with PI and CPS3, on the contrary to existing theory. A detailed discussion on the experimental results are provided in the next chapter along with the major limitation of this work and suggestions for future work to further improve the developed set-up for Subharmonic Imaging of the PVA-based UCAs.
Chapter 4

Discussion

The backscatter enhancement provided by UCAs through conventional B-mode techniques results in poor diagnostic capabilities for important vascular components due to strong echoes from surrounding tissues. Imaging techniques that offer the possibility of separating the nonlinear behavior of the agents from the linear behavior of surrounding tissue must be exploited for improved efficiency. One may even further enhance this efficiency by focusing on the subharmonic generation of UCAs, which is an even more exclusive property of the agents than the generation of higher harmonics, for improved CTR. Superior tissue separation as well as the reduced attenuation, due to the lower frequency of the subharmonic component, contribute to a more robust technique for imaging deep vascular targets in the neighborhood of highly echogenic tissues. This work addressed the design and development of a one-probe setup for nonlinear imaging of polymeric based PVA agents using The Verasonics Research System, with special focus on nondestructive Subharmonic Imaging. The obvious benefits of the PVA agents, such as high stability and prolonged shelf-life, makes them a promising choice for various applications but the stiff shell limits their acoustic performance in comparison with traditional lipidic agents [3].

The setup was developed according to findings in previous studies as well as in accordance with the requirements and limitations of The Verasonics Research System. Different imaging techniques were implemented and tested with and without a focus on the subharmonic component of the backscattered signals by the use of a LBF. The performance of each technique was determined in terms of CTR and CNR.

The backscattered enhancement, assessed by CNR, provided by the PVA contrast agents through the FB technique resulted in 22.98 dB. These results demonstrate the capabilities of the PVA agents of improving the echogenicity of the suspension that bears the agents (PVA+degassed water) in contrast to the suspension that does not bear the agents (degassed water). This enhancement strings along with previous studies of polymeric based contrast agents which have reported an enhancement of the order of 20 dB [4, 22–24]. However, the limitations of the FB technique are obvious in terms of CTR. The FB technique provided a CTR of only 5.53 dB, although in a stationary tissue mimicking environment. These findings suggest that, even in somewhat ideal agent-detectable conditions, the performance of the FB technique is unsatisfactory.

The image performance of the PI technique resulted in a CTR of 16.08 dB and a CNR of 36.83 dB. This was an improvement of 10.55 dB and 13.85 dB, respectively, in comparison with the FB technique. As can be seen by comparing Figures 3.2a and 3.2c, the PI provided a significant tissue suppression at the fundamental frequency (6.92 MHz) compared with the FB technique while preserving majority of the signals produced by the PVA agents. This is the result of the additional PVA excitation provided by the PI technique (two transmission pulses instead of one) in addition to the powerful suppression of linear tissue echoes.
The improved CNR corresponds to the improved suppression of linear noise while preserving relatively more signals from the PVA agents. The fundamental drawback of the PI technique in comparison with the FB technique was, as expected, the reduction in frame rate by a factor approximately 2 due to the implementation of two transmissions for each scan line.

The CPS3 technique resulted in further improvements and performed best of the six techniques in terms of CTR. The CPS3 provided a CTR of 19.52 dB and a CNR of 36.14 dB. In comparison with the PI technique, this is an improvement of 3.44 dB in CTR while the CNR did not result in any significant difference in terms of the standard errors. The CPS3 combined the principles of PI and AM in a three pulse configuration. Previous studies have shown the benefits of similar configurations over simple PI or simple AM due to the fact that both the phase and the amplitude of the transmitted signals can be varied between successive pulses. This in turn increases the possibility of canceling out linear signals provided by tissue, allowing greater focus on nonlinear signal generation by the agents [3]. Also, the amplitude spectra for both PI and CPS3 (Figures 3.2c and 3.2e) suggest a considerable generation of nonlinear signals around the fundamental frequency component (∼ 5-9 MHz). The broad band peak of the PVA agents (Figure 3.2a) is only suppressed on average approximately 10 dB (Figures 3.2c and 3.2e) while the broad band peak of the tissue is suppressed between 16 and 30 dB. However again, the increased amount of transmissions for each scan line reduced the real-time imaging capabilities of the configuration and the CPS3 and CPS3/LBF performed worst in terms of frame rate compared with the other techniques. Compared with the FB imaging technique, the frame rate dropped by a factor of 3 due to the transmission of three transmissions instead of only one for each scan line. Another drawback of the CPS3 and a common drawback with all multi-pulse techniques is that they suffer from motion artifacts if rapid motions take place between the successive pulses that make up a scan [11]. Thus, the time between successive pulses needs to be minimized to better prevent motion artifacts.

The implementation of the LBF around the subharmonic component (3.46 MHz) combined with the FB technique resulted in an improvement of about 1.50 dB in terms of CTR and CNR compared with the simple FB. However, the LBF combined with PI and CPS3 resulted in a poorer performance in comparison with simple PI and simple CPS3, respectively. On the contrary to these results and as previously mentioned, former studies have suggested that imaging at the subharmonic frequency is a superior method to imaging at higher harmonics where CTR can be maximized due to the fact that subharmonics are exclusively generated by MBs [25–27]. There are few things that are worth discussing in relation to why this improvement was not achieved in the PI/LBF and CPS3/LBF technique in the setup of this work. First, referring to Figure 3.2a, the majority of the signals produced by the tissue are contained on a relatively broad band instead of having a distinct peak at the fundamental frequency of 6.92 MHz. The averaged tissue spectra shows a considerable amount of signals at the subharmonic frequency. This is believed to be the result of spectral leakage as well as non-specific frequency transmission in the transmitted pulses due to relatively short pulses in the time domain. The amplitude spectrum suggests that the nonlinear behavior of the PVA agents was quite insufficient, producing only about 8-10 dB enhancement above the tissue over a broad band of frequencies. Thus, even after implementing the multi-pulse techniques of PI and CPS3 where the fundamental component of tissue is suppressed to the order of about 20 dB, the enhancement of the subharmonic component of the PVA over the subharmonic component of the tissue is not acceptable for improved CTR. Also more importantly, regarding Figures 3.2a, 3.2c and 3.2e, one can clearly see that both PI and CPS3 mainly suppress tissue signals at the fundamental frequency and higher frequencies, while tissue signals around the subharmonic component are barely suppressed relative to the PVA signal suppression. So, by implementing the LBF,
the distinct enhancement of the PVA agents over the tissue around the fundamental component is lost without being compensated for by acceptable subharmonic CTR. These findings suggest that further improvements must be implemented to suppress these limitations and for developing a more efficient setup for subharmonic imaging, as will be discussed in the following section.

4.1 Limitations and Future Work

To further extend the quality of the setup which was developed in this work, the major limitations need to be addressed. The major limitations included among others the transducer choice, the capability of the pulse technique to suppress tissue signals at frequencies below the fundamental frequency as well as the transmission frequency.

To begin with, the narrow bandwidth of the Philips ATL L7-4 transducer which was used in the setup may have had too much impact on the experimental results. The upper limits of the bandwidth, 7 MHz, prevented transmitting with sufficient quality closer to the average resonance frequency of the PVA agents, between 8 and 9 MHz. Despite providing nonlinear images of acceptable quality through PI and CPS3, this may have lead to insufficient subharmonic generation provided by the agents for competent subharmonic images. Also, the subharmonic component (3.46 MHz) of the transmitted signals was below the lower limits of the transducer’s bandwidth, 4 MHz, which limited the sensitivity of the probe to subharmonic signals. Attempts were made to include the subharmonic component within the bandwidth of the probe which resulted in the transmitted frequency component being raised above the upper bandwidth limit. This however resulted in degraded image performance. A suggestion for future work is to adapt the current setup for a broader bandwidth probe such as the 256 channel L12-5 transducer. The transmitted frequency and the subharmonic frequency should ideally be well within the -6 dB bandwidth limits of the probe which suggests an absolute minimum bandwidth of 75%. However, while broader bandwidth probes allow for improved resolution, it comes at the cost of decreased sensitivity [28].

The multi-pulse techniques of PI and AM have been implemented in previous studies for subharmonic imaging for improved tissue suppression and decreased spectral leakage [9–11, 29, 30]. However, for the setup of this work, the PI and the CPS3 did not provide sufficient tissue suppression at lower frequencies despite providing a significant suppression at the fundamental and higher frequencies. In order to further improve the configuration of this work, if signal generation from tissue at subharmonic frequencies can not be prevented or at least minimized, a CPS design which performs better in suppressing these lower-region frequencies from the tissue needs to be carried out. Previous studies, such as have reported contrast pulse sequences which isolate a particular harmonic by suppressing all other components [31]. However, to the author’s best knowledge, none have been developed specifically for subharmonic isolation.

There are in practice two categories of subharmonic spectral components, classified here as TR and T2R. The TR way transmits ultrasound pulses at the resonance frequency of the MBs, $f_r$, while focusing on the subharmonic component at half the resonance frequency, $f_r/2$. The T2R method transmits the pulses at two times the resonance frequency of the bubbles, $2f_r$, while focusing on the subharmonic generation at the resonance frequency, $f_r$. For this work, the TR method was chosen since the resonance frequency of the PVA batch was at 7-10 MHz and because of the transducer used. If the T2R method would be implemented with the PVA agents, the transmission requires a frequency of 14-20 MHz which may be too high for clinical
applications and when targeting deep areas due to the rapid increase in attenuation. However, the major benefit of the T2R method is that the pressure threshold for subharmonic generation is at its absolute minimum and is much lower than for the TR method [11, 26]. Previous findings have also stated that the pressure threshold for subharmonic generation when using the TR method is above the destruction threshold of the MBs [26]. This is a concern for the future work of nondestructive subharmonic imaging of PVA-shelled agents where the trade-off will be between sufficient subharmonic generation, no bubble destruction and acceptable attenuation in tissue. A suggestion for future work is to better control the size distribution of the PVA agents in order to acquire larger MBs. This in turn should lower the resonance frequency of the agents for T2R imaging, meaning lower transmission frequency requirements and lower attenuation [2]. Then, by choosing a suitable transducer the previously mentioned trade-off may reach an acceptable state.

At last, also worth addressing is that, despite offering a flexible design of developing ultrasound imaging sequences and processing, The Verasonics Research System brought about few limitations due to system requirements. These limitations included the requirements set on the filter design, sampling frequency and transmit waveform. The filter requirements (Section 2.4) prevented the design of an optimal filter around the subharmonic component. The sampling frequency requirements (Section 2.2) limited the possibility of detecting agent performance at the second harmonic. Finally, the envelope shape of the parametric waveform (Section 2.4) was not be altered thus different envelopes could not be developed for improved performance, such as the chirp pulse excitation. The frequency modulated chirps have been reported to be more robust to the distortion which is caused by the frequency-dependent attenuation as ultrasound propagates through soft tissue [11]. Also, to prevent the broad frequency response obtained from the tissue (Figure 3.2a) and thus minimizing tissue frequency components at subharmonic frequencies, one may want to implement long duration chirps. This will increase the total energy of the system resulting in an improved Signal-to-Noise ratio as well as an improved frequency specificity of the transmission. Thus, one may expect a more distinct peak at the fundamental frequency from tissue and decreased signal power from other frequencies, such as at the subharmonic frequency. The reduction in spatial resolution due to the long pulses can then be recovered by the implementation of a matching filter on the receiving side which reduces the initial duration of the chirp pulses [11]. A final suggestion for future work is to exploit the possibility of altering the filter design and the waveform design at run-time through an external function according to the previously mentioned properties for improved set-up specific to Subharmonic Imaging.
Chapter 5

Conclusion

Three different ultrasound imaging sequences were implemented on The Verasonics Research System. Two of those, PI and CPS3, were implemented for focusing on the nonlinear generation provided by air-filled, PVA-shelled UCAs. Additionally, the same sequences were implemented with a LBF over the subharmonic component for subharmonic specific imaging. The FB technique, which mainly served as a base technique for comparison purpose, showed that the PVA agents provided significant backscatter enhancement; 23 dB in terms of CNR, while the performance in terms of CTR was poor due to high amount of tissue signals. The development of the nonlinear imaging techniques of PI and CPS3 was successful. Both techniques provided significant tissue suppression while still preserving majority of the signals from the PVA agents, which resulted in improved CTR and CNR. Of the six different techniques developed on The Verasonics Research System, the CPS based on a combination of PI and AM in a three pulse configuration, CPS3, performed best in terms of CTR. The subharmonic specific imaging through the use of a LBF with PI and also CPS3 resulted in degraded image performance due to high amount of tissue signals around the subharmonic region, which was not suppressed by the PI and the CPS3, as well as due to the poor detection of subharmonic signals from the PVA agents. For an improved subharmonic imaging configuration, one must limit the tissue backscattering at subharmonic frequencies or develop a CPS which enables a more efficient suppression of these tissue components. In addition, attempts should be made to allow for the T2R transmission configuration (Chapter 4) for an enhanced subharmonic generation at lower pressure thresholds as well as to make way for the ability of the system to generate long duration chirp transmissions with matching filters for more robust and frequency-specific transmissions.
Bibliography


Appendix A

State of the Art

The success of ultrasound imaging as a diagnostic tool has mainly been a result of its ability to produce quality images in real time. Also, its portability, safety and low cost compared with other imaging modalities makes it the optimal imaging choice for various clinical applications [32]. However, in conventional ultrasound imaging, the detection of blood and blood flow within vessels in the neighborhood of highly echogenic\(^1\) tissue is known to be highly insufficient. This is mainly due to the fact that the echoes produced by red blood cells at diagnostic frequencies (2-15 MHz) are very weak in comparison to the surrounding tissues, up to 10,000 times weaker, resulting in blood echoes to lie outside the dynamic range of the ultrasound display. In Doppler imaging, similar problems arise when imaging small vessels, such as the supplying vessels of the myocardium. The weak echoes are difficult to detect due to the strong echoes from surrounding tissue and the velocity of the blood flow in the small diameter vessels is too low to produce detectable Doppler shift. Thus, to overcome these problems, one may want to either increase the blood echo sufficiently above the neighboring tissues, or to be able to suppress the echoes from these surrounding structures and by so introducing the blood echo into the dynamic range of the ultrasound display [1]. This is where the introduction of ultrasound contrast agents (UCAs) into the blood pool gives rise to new possibilities.

A.1 Contrast Enhanced Ultrasound

During the past three decades, Contrast-Enhanced Ultrasound has expanded and evolved from being entirely an investigative tool to a routine diagnostic procedure [33]. Contrast-Enhanced Ultrasound (CEUS) comprises the application of ultrasound contrast medium to the conventional sonography. The interest in developing UCAs to serve as the contrast medium for clinical applications commenced after the earliest observations of Gramiak and Shah in 1968 [5]. Gramiak and Shah observed cloud of bubbles after an intra-aortic catheter injection of saline [34]. They noticed that the introduction of the freely floating air bubbles into the blood pool, following the injection, improved the blood echogenicity. Five decades later, the development of UCAs for diagnostic purposes is still of great interest within the field. Today, the application of UCAs is exclusively approved for diagnostic imaging but recent development in therapeutic options, such as site-specific drug delivery, has opened additional possibilities for future applications [5].

\(^1\)The ability to reflect sound waves.
A.2 Ultrasound Contrast Agents

The fundamental role of UCAs for diagnostic purposes is to enhance the scattering efficiency of ultrasound by the tissue that bears them, leading to an improved Signal-to-Noise ratio (SNR). Red blood cells are poor reflectors of ultrasound due to the fact that their acoustic impedance\(^2\) is essentially identical to that of the surrounding blood plasma and hence almost no echoes are produced. By injecting customized UCAs, into the blood pool, usually via intravenous administration, a strong discontinuity in the acoustic impedance of the tissue is introduced, leading to great improvement in the blood pool’s echogenicity [5, 6].

UCAs have been commercially available since 1991. In general, they compose of gas-filled microbubbles (MBs) with diameters of 1-10 µm and an average diameter of 2-3 µm. In comparison, the average diameter of red blood cells is 6-8 µm [2]. The size restriction on the MBs follows the requirement that the MBs must be small enough to pass through the smallest capillaries but too large to extravasate out of the vessels or to be filtered out by the lungs [9]. The first UCAs consisted of freely floating gas bubbles and their lifetime after intravenous administration was only a few seconds. UCAs in modern-use compose of low-diffusive gases encapsulated in a 10-200 nm thick biodegradable shell for increased stability which provides acceptable lifetime of the bubbles for diagnostic purposes [3, 8]. Prominent gases in current use are nitrogen or per-fluorocarbon while the protective shells usually consist of phospholipids, albumin, or polymers. Table A.1 provides specifications of three commercially available agents licensed for cardiology applications in the United States and the European Union [8].

<table>
<thead>
<tr>
<th>Name</th>
<th>Shell Material</th>
<th>Gas</th>
<th>Mean Size (µm)</th>
<th>Imaging Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optison(^{TM})</td>
<td>Albumin</td>
<td>(C_3F_8)</td>
<td>2.0-4.5</td>
<td>2.5-4.5</td>
</tr>
<tr>
<td>Definity(^{®})</td>
<td>Phospholipid</td>
<td>(C_3F_8)</td>
<td>1.1-3.3</td>
<td>2-10</td>
</tr>
<tr>
<td>SonoVue(^{®})</td>
<td>Phospholipid</td>
<td>(SF_6)</td>
<td>2-8</td>
<td>3-6</td>
</tr>
</tbody>
</table>

A.3 Acoustic Response of Ultrasound Contrast Agents

The acoustic response of the UCAs is highly dependent on the physical properties of the gas core and the protective shells that make up the MBs [2]. Exposed to a beam of ultrasound, the MBs react to the oscillating pressure field with volume pulsations when the wavelength of the transmitted ultrasound is much larger then the size of the MBs (Figure A.1). The MBs will expand and contract due to the constant switching of positive and negative pressure regions of the ultrasound wave (Figure A.1).

In the negative pressure region (rarefaction), the bubbles will expand while in the positive region (compression), the bubbles will contract. These periodic changes in the MBs radius suggest, just as vibrations in other structures, that these oscillations carry a resonance frequency at which the MBs will both scatter and absorb ultrasound with particularly high efficiency [1].

\(^2\)The product of density and speed of sound in the material [2].
The importance of the resonance phenomena of the MBs is especially significant due to the fact that the resonating MBs will also themselves serve as a source of sound, that radially radiates from their location, instead of serving only as passive reflectors due to the acoustic impedance mismatch between the encapsulated gas and the blood plasma [7]. The resonant frequency of a certain MB depends mainly on the MB radius, but also the physical properties of the shell and the compressibility of the gas core. Experimental findings have demonstrated that the resonant frequency is inversely proportional to the MBs radius. The stiffness of the surrounding shell increases the resonance frequency while its viscosity increases damping [2]. It is of great importance, and somewhat coincidence, that the UCAs in past and current use have their resonance frequencies well within the frequency boundaries of a conventional clinical ultrasound scan [1].

### A.4 Three Regimes of Acoustic Behavior

The signature reaction of the MBs in a field of ultrasound can be divided into three major regimes in terms of the acoustic intensity of the field. The Mechanical Index (MI) is, in practice, the index of quantifying the intensity of the transmitted ultrasound beam. The MI is also known to be the primary determinant of how the UCAs behave in the field of ultrasound and is one of the most important parameter in a contrast echo study [1]. The Mechanical Index is defined as the peak negative pressure, \( P_{\text{neg}} \) (Equation A.1), divided by the square root of the ultrasound frequency, \( f \). The index relates to the mechanical work that is applied to a target of interest during a half cycle of sound [35].

\[
MI = \frac{P_{\text{neg}}}{\sqrt{f}} \tag{A.1}
\]

Table A.2 presents an example of the three major MI regimes for a specific microbubble exposed to a field of ultrasound at 1.0 MHz. The margins of the intervals depend on the physical properties of the MBs, among other factors.

<table>
<thead>
<tr>
<th>MI Regime</th>
<th>Peak Pressure (example)</th>
<th>MI @ 1MHz</th>
<th>Bubble Behavior</th>
<th>Acoustic Behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear</td>
<td>&lt; 100 kPa</td>
<td>&lt; 0.1</td>
<td>Linear Oscillation</td>
<td>Backscatter Enhancement</td>
</tr>
<tr>
<td>Nonlinear</td>
<td>100 kPa - 1 MPa</td>
<td>0.1 - 1.0</td>
<td>Nonlinear Oscillation</td>
<td>Harmonic Backscatter</td>
</tr>
<tr>
<td>Destruction</td>
<td>&gt; 1 MPa</td>
<td>&gt; 1.0</td>
<td>Disruption</td>
<td>Transient Harmonic Echo</td>
</tr>
</tbody>
</table>

At low acoustic pressure, the lowest MI regime, the volume pulsations of the MBs act in a linear manner. That is, the backscattered ultrasound from the MBs holds essentially the same frequency as the transmitted frequency, \( f_0 \) [8]. The MBs therefore "only" serve to produce backscatter enhancement [1].

Exposing the MBs to higher incident pressure (the intermediate MI regime), the resonant oscillations of the bubbles can be induced to nonlinear oscillations. That is, the expansion and contraction are not equal [1]. In principle, the MBs expansion is unlimited while the compressibility is not [2]. This is due to the fact that when the bubble is compressed sufficiently
much, it becomes stiffer and resists further reduction in its radius. In the rarefaction region, the bubble becomes less stiff and therefore increases its radius much more in comparison [1].

In other words, the MBs resist compression much more than expansion [3]. This phenomena gives rise to the possibility of harmonic mode production [1]. The generation of subharmonics backscattering \( (f_0/2) \), and higher harmonics scattering (e.g. \( 2f_0 \), \( 3f_0 \), etc., Figure A.2), provided by the nonlinear oscillations of the MBs, can be considered as the signature of the MBs in a way that they differ from those reflected from surrounding tissues. That is, the tissue’s linear backscattering will essentially contain the fundamental frequency, \( f_0 \), while the MBs’ nonlinear backscattering may also contain subharmonics and higher harmonics. This introduces the possibility of separating the UCAs-bearing tissue from the surrounding tissues, as will be discussed in later stages of this section [8].

The third MI regime, is introduced when the MI is raised to a level that results in MBs destruction. The disruption of the MBs results in short pulses of transient harmonic echoes [1].

It is worth mentioning that the borders between these intervals are, in practice, not always very clear or sharp due to the differences in sizes in a conventional bubble population and because of spectral overlap [1]. Modern contrast-enhancing ultrasound techniques drive the MBs at the relevant interval that is clinically and diagnostically advantageous for their intended purpose. The following sections discuss some of the most relevant imaging techniques that utilize ultrasound contrast agents, grouped according to Table A.2. Short introduction on each imaging method will be included as well as a discussion on how the the UCAs benefit the particular technique and what are the major challenges and limitations.

### A.4.1 Imaging Techniques: Linear Oscillation

As previously discussed, exposing UCAs to an ultrasound field in the lowest MI regime results in linear backscattering. The fundamental benefit in the linear regime arises from the fact that the MBs introduce a high discontinuity in the acoustic impedance between the blood plasma and the MBs. This in turn greatly increases the echogenicity of the tissue as a whole.

In short, Conventional B-mode Imaging simply displays the backscattered echoes that are produced when the transmitted ultrasound wave experiences acoustic impedance mismatch at interfaces, as it travels through matter. Larger acoustic impedance mismatch results in higher amplitude of the echo. The amplitude of the received echo then determines the displayed brightness of the corresponding interface location [36]. As mentioned before, the weakness of the echoes from blood arises from the cells themselves which are poor scatterers of an
ultrasound wave. The acoustic impedance mismatch of a typical MB in the blood pool compared with a blood cell (in the blood pool) has been reported to be as high as $10^{14}$ or $140 \text{ dB}$. However, after dilution of the MBs, the real increase in the scattering power is much lower, or around 20 dB. This increase in echogenicity is still of immediate benefit in B-mode imaging at the linear regime for various purposes, such as for improved estimation of ventricular volume and output as well as better delineation of the wall of the ventricular chamber. On the other hand, for Conventional B-mode imaging and linear backscattering, this benefit is limited to large volumes and high concentrations of MBs [37].

When imaging vessels in the presence of a highly echogenic tissue, such as the supplying vessels of the myocardium, the echoes provided by the UCAs are still some 20 dB below the strong echoes from the tissue. One may ask if we could not raise the concentration of MBs to a level where sufficient increase in echo is reached. Unfortunately, a trade-off exists between the concentration of MBs and attenuation of the ultrasound wave. Thus, at high MBs concentration one cannot image deeper targets and a sharp rise in attenuation may introduce shading artifacts of deeper regions [37]. Thus, in the presence of tissue, for blood and blood flow diagnosis, Contrast-Enhanced B-mode Imaging at low Mechanical Index results in poor UCAs detection [7]. Alternative methods are needed, as will be discussed in chapter A.4.2.

In Doppler Imaging, the Doppler effect gives rise to the imperative capability to realize and quantify blood flow. The scattering echoes contain information about the velocity of flow in a volume of interest and the Doppler shift allows for evaluating the direction of the flow. The 20 dB increase in the backscattered echo strength, introduced by UCAs, has been of benefit for Conventional Spectral Doppler. The increased echo strength provided the detection and measurements of the blood flow in vessels that before were too deep to analyze. In Color Flow Imaging (CFI) a conventional B-mode image is superimposed on a color map where the color of each pixel is governed by the mean Doppler shift for that particular location. The color intensity of each pixel in CFI is fixed and only the color itself presents the blood velocity and direction. In Power Doppler Imaging (PDI), each pixel’s color intensity is governed by the mean power of the Doppler signal at that particular location and takes no interest in the amount of Doppler shift. PDI therefore indicates the amount, or number, of moving blood cells at a volume of interest. The use of UCAs in both CFI and PDI provides better signal to noise ratio due to increased echo strength from the blood pool [37].

Limitations of using UCAs in CFI, in addition to the identical limitations in B-mode imaging, is the so-called "blooming" effect where the full brightness color spreads outside the vessel walls. This is mainly caused by MBs that lie against the edges of the walls and their high sensitivity. They in turn produce detectable echoes even though the center of the particular pulse lies outside the vessel. PDI suffers much less from the blooming effect since they display echoes outside the vessels with less brightness while in CFI all pixels carry the same brightness, only different colors. PDI directly shows the concentration of MBs in the volume of interest and the PDI visualization of showing only one color with different brightness aids the eye in appreciating the different flow velocities. The major limitation of using PDI with UCAs is the "flash" artifact. Again, the presence of moving and highly echogenic tissue interrupts the detection of the slowly moving blood. Imaging flow in large vessels has proved to be relatively efficient using PDI but the smaller vessels can not be efficiently detected due to the low velocity of the blood they retain and the high echogenicity of surrounding tissue [37].
A.4.2 Imaging Techniques: Nonlinear Oscillation

When UCAs were first introduced, the general believe was that the improved ultrasound scattering by the blood pool, produced by the immense increase in acoustic impedance mismatch by the MBs, would provide sufficient contrast by conventional imaging methods. By doing so, the target of interest should exhibit enough enhancement so that its grayscale level would provide acceptable contrast [7]. As previously discussed, this was and is not the case. For small vessels and deeper areas, the enhancement still leaves the target of interest well below the echoes from the surrounding, highly echogenic tissues. Because of these limitations, later techniques were designed to incorporate bubble-specific imaging by focusing on their unique ability to produce harmonics.

The harmonics produced by the nonlinear behavior of MBs in the intermediate MI regime give rise to the possibility of distinguishing the agents from the surrounding tissues [1]. The foundation of that ability is that the harmonics produced by the MBs are produced in much lower amount by tissue and can therefore be regarded as the MBs signature. Thus, one aims at transmitting at one frequency and focusing on the harmonics, the MBs signature, for image reconstruction. The detection of microvessels, bearing UCAs, has been shown to be possible using harmonic imaging, even if the microvessels are in the presence of highly echogenic tissue, such as the myocardium [1].

Four techniques which serve to isolate the subharmonic component or higher harmonics in the backscattered signals will be discussed. Those are (1) Filtering, (2) Pulse Inversion, (3) Contrast Pulse Sequence Based on Three Pulses, and (4) Chirp Contrast Pulse Sequence Based on Three Pulses.

Filtering

In nonlinear imaging, given that the MBs are exposed to a sufficiently high MI for harmonics production, a specific harmonic component or a set of harmonic components can be isolated through filtering. Depending on the application, one can implement high-pass, low-pass or band-pass filtering operations on the backscattered signals. The fundamental frequency component can be suppressed, resulting in an increased relative strength of the harmonics of interest. This enables improved detection of UCAs-bearing structures, such as small vessels that were not visible before. The main drawbacks are the loss of sensitivity, since most of the energy from both the MBs and the surrounding tissues are essentially in the fundamental frequency band [37]. Also, the purpose of transmitting at one frequency and receiving at a specific harmonic is to appreciate the tissue that bears the UCAs. However, the detected harmonics may not be entirely from the MBs due to the small production of harmonics by the surrounding tissues, the so-called tissue harmonics. Just like for MBs harmonics, tissue harmonics emerge because of asymmetry but through a slightly different mechanism; the sound travels slightly faster through matter in the compression phase of the wave cycle in comparison with the speed in the rarefaction phase. This results in a distortion of the shape of the wave. Even though this effect, usually referred to as finite amplitude distortion, is relatively small, it is sufficient to produce detectable tissue harmonics and explains why solid tissue does not appear completely black in harmonic imaging [1]. These limitations have been overcome to a large extent by the use of multi-pulse techniques, as will be discussed in the following sections [37].
Pulse Inversion

The Pulse Inversion (PI) technique is recognized as one of the most promising method for improving the performance of nonlinear imaging. The main advantage of the PI compared with filtering techniques is the ability to reduce potential interference from the linear component of the acoustic signal. Additionally, though filtering techniques alone can provide acceptable efficiency for nonlinear imaging, it suffers from spectral leakage which in turn leads to poorer contrast. The spectral leakage can be greatly reduced with the PI technique [6].

The fundamental principle of the PI technique is simple (Figure A.3). The technique requires the transmission of two ultrasound pulses, one after the other, for each acoustic beamline. After the echoes from the first pulse have been received, an inverted version of the first pulse is transmitted. In principle, the linear component of the received echoes should be the same except that one is the inverted version of the other. On the other hand, due to the random behavior of the nonlinear components, the nonlinear components of the received echoes from the two pulses should give rise to dissimilar shapes. Thus, by summing up the two transmissions, one can suppress the linear component while holding on to the harmonics. Thus, the main function of the PI technique is to suppress the fundamental component and improve the visualization of the MBs signature [6].

A similar configuration is the Amplitude Modulation (AM) (Figure A.3). The principle of AM is similar to PI. Two or more pulses of different amplitudes are transmitted and the backscattered signals stored. Weighting factors are then implemented on the received echoes to compensate for the different amplitudes. The received signals from each pulse are then subtracted. Like for PI, this leads to the suppression of linear signal components while nonlinear signals will be distorted to different extent and thus not canceled.
The most obvious limitation of the PI ans AM technique is that temporal resolution (frame rate) becomes poorer because two transmissions are needed for each scan line. An alternative method to overcome this problem is to implement e.g. the PI technique on alternative scan lines. That is, a positive polarity pulse is transmitted on a scan line and then on the adjacent scan line (instead of the same scan line), an inverted pulse is transmitted. The echoes received by adjacent scan lines are summed to cancel out the linear component of the echoes. However, though this method improves temporal resolution, it suffers from slight imperfections in fundamental cancellation since the origin of the two pulses is not the same [37].

**Contrast Pulse Sequence Based on Three Pulses (CPS3)**

The Contrast Pulse Sequence based on three pulses (CPS3) is one of the most widely used contrast pulse methods. The principle of the technique is to transmit three pulses for each scan line. In general, the amplitude coefficients of the three pulses are scaled as 0.5, -1, and 0.5. The received echoes are then summed up to cancel out the linear component. CPS3 has shown to produce greater tissue suppression than the PI technique [3]. The CPS3 method can be regarded as a combination of the PI method and Amplitude Modulation (AM). By choosing suitable weighting factors for the amplitudes and the phase of the three pulses, one may isolate a particular harmonic component by suppressing all other components [37]. Compared with PI, the CPS3 method is known to provide greater robustness against noise and motion artifacts [3]. However, it suffers from poorer temporal resolution due to the amount of pulses for each scan line.

**Chirp Contrast Pulse Sequence Based on Three Pulses (Chirp CPS3)**

The Chirp CPS3 method utilized the same principle as CPS3 but instead of sending three traditional continuous wave (CW) pulses it uses three long duration linear chirp pulses. A matched filtering is then implemented to compress the long chirp pulses for improved axial resolution. The Chirp CPS3 has shown to provide an even better UCAs detection than the PI, AM and CPS3 methods [3, 11]. Table A.3 summarizes the four multi-pulse techniques previously discussed.

<table>
<thead>
<tr>
<th>Method</th>
<th>Pulse Waveform</th>
<th>Transmission Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI</td>
<td>2 CW pulses</td>
<td>+1 -1</td>
</tr>
<tr>
<td>AM</td>
<td>2 CW pulses</td>
<td>+1 +0.5</td>
</tr>
<tr>
<td>CPS3</td>
<td>3 CW pulses</td>
<td>+0.5 -1 +0.5</td>
</tr>
<tr>
<td>Chirp CPS3</td>
<td>3 Chirp pulses</td>
<td>+0.5 -1 +0.5</td>
</tr>
</tbody>
</table>

Table A.3: Summary of the four multi-pulse techniques. Adapted from [3].
A.4.3 Imaging Techniques: Destruction

Modern contrast-enhancing techniques involved in the high MI regime of transient disruption (Table A.2) incorporate the principle of detecting high, transient spikes in the received echoes. The disruption of the MBs results in the release of high energy which can be utilized for MB detection. High-MI techniques provide better sensitivity to MB detection but increase the risk of cavitation due to the high acoustic output. [37].

Release Burst Imaging is a technique which utilizes the disruption of MBs. First, a high-MI pulse is transmitted, which serves to rupture the MBs. This is followed by a few sets of low-MI imaging pulses that serve to detect the transient MBs fragments as well as the free gas bubbles that are released from the gas core. Additional application in the destructive regime is the site-specific drug delivery where the MBs are disrupted at a relevant location and release drugs from their gas core [37].

A.5 Subharmonic Imaging

In more recent years, the interest in a new bubble-specific imaging technique has expanded rapidly. The principle of Subharmonic Imaging (SHI) is that UCAs are able to generate signals at half the fundamental transmission frequency. In ultrasound imaging, this ability is exclusive to UCAs [32]. The two fundamental requirements for efficient SHI and optimal image contrast are: (1) to maximize subharmonic signal-to-noise ratio from the UCAs, and (2) to minimize subharmonic detection from non-UCAs-bearing structures [20]. Transmitting at a fundamental frequency $f_0$ and focusing on $f_0/2$ component has been reported to improve visualization of UCAs within the vascular system due to the suppression of echoes from surrounding tissues [32]. In the previously mentioned Harmonic Imaging modes (e.g. transmitting at $f_0$ and receiving at $2f_0$ or $3f_0$) a full tissue suppression cannot be achieved (f.x. by PI) because of the tissue harmonics from surrounding structures. This results in echoes from the surrounding tissues at the second harmonics that one would ideally not want to display. On the other hand, SHI has shown to do a better job than HI in tissue suppression and an improved real time visualization of contrast-enhanced blood flow [32].

It has been reported that the strength of the subharmonic signal is greatest when the transmission frequency, $f_0$, is two times the natural resonance frequency, $f_r$, of the MBs (Figure ??) [37]. The subharmonic amplitude generated by MBs as a function of acoustic pressure has been reported to show an S-curve behavior; at low MI the SH component is insignificant, at the growth stage the SH component increases with increased MI, and at high enough MI the SH amplitude saturates [20]. Number of cycles per pulse has also showed similar behavior; for short pulses (e.g. <16 cycles) the SH signal grows with increased cycles per pulse but then saturates at longer pulses [20]. Compared with higher harmonics imaging, the strength of the subharmonic spectrum is higher than of the second harmonic [37]. Also, the tissue harmonics which limit somewhat higher harmonics separation, are not generated (at least much less generated) at the subharmonic frequency band [20]. Another benefit is that the receiving subharmonic component is of lower frequency than the fundamental component and the higher harmonics, which results in lower attenuation of the subharmonic component of the ultrasound beam. One should thus be able to image deeper regions with SHI in comparison [37]. On the other hand, because the transmitted frequency can be higher than in fundamental or HI, since receiving is at $f_0/2$, one can expect better lateral resolution and less risk of cavitational bioeffects [20]. Shankar et al. reported in a comparative study on SHI and 2nd
HI that SHI may be a superior method for contrast imaging [25].

The main limitation of SHI with UCAs, which originates from the improved tissue suppression, is that we lose the visibility of important anatomical landmarks. The landmarks are an important feature of the acquired image to be able to better locate cardiovascular activity within a tissue or an organ (or lesions). In other words, we can almost only see the blood flow behavior, but not the tissue or the organ that the vessels are supplying. To overcome this problem, two methods have been used. Firstly, to acquire a grayscale image prior to the SHI to detect landmarks and "fuse" the frames together in post-processing. However, this method suffers from an increased scan-time and introduces uncertainties due to possible motions between the different scans. The other method involves a dual grayscale-SHI technique where the two techniques are implemented simultaneously. A grayscale image captures the tissue landmarks within a region of interest (ROI), while at the same time the SHI provides information on blood flow behavior [32]. Another limitation comes from the fact that the strength of subharmonic signals increases with longer pulses (more cycles per pulse). This introduces a trade-off between the strength of the subharmonic signal and axial resolution, because axial resolution becomes poorer with longer pulse duration [37].

Different techniques have been implemented to provide efficient subharmonic detection for contrast imaging such as the above mentioned multi-pulse techniques (PI, AM, CPS3) combined with suitable filtering operations. However, SHI is a relatively recent field in contrast imaging and further development is being carried out for necessary improvements. Past and present evidence indicate that SHI might be the optimal method for various applications in contrast enhanced ultrasound imaging.