Ultraharmonic imaging of polymer-shelled microbubbles

Ultraharmonic-avbildning av mikrobubblor med polymerbaserade skal

DIMITRIOS EVANGELOU
Abstract

Ultrasound has been established as one of the most widely used imaging modalities for diagnostic purposes, due to the several advantages it provides in comparison with other techniques. Hence, ways to further improve the confidence in diagnoses provided by ultrasound are constantly being investigated. One of them is the introduction of Ultrasound Contrast Agents, which can enhance the weak echoes produced by the small vessels, improving the imaging performance.

In this study, a setup was created and six ultrasound imaging techniques were implemented by using the Verasonics Research System®, in order to take advantage of the different behavior between the tissue and the Polyvinyl-Alcohol microbubbles, when exposed to ultrasound. These were: Fundamental B-mode, Ultraharmonic, Pulse Inversion, Subharmonic Pulse Inversion, Ultraharmonic Pulse Inversion, Combination of the Sub- and Ultraharmonic Pulse Inversion.

For the assessment of the bubbles’ response, the amplitude spectra were used, which showed a limited detection around the ultraharmonic region. For the evaluation of the imaging performance of the techniques, the Contrast-to-Tissue (CTR) and Contrast-to-Noise Ratios (CNR) were calculated. The Combination of the Sub- and Ultraharmonic Pulse Inversion reported the highest imaging performance among all the techniques. A comparison with previous articles provided a similar pattern in terms of CTR.

Keywords

Subharmonic Imaging, Ultraharmonic Imaging, Polyvinyl Alcohol, PVA, Verasonics, Ultrasound Contrast Agents, Non-linear imaging, Contrast-to-Tissue ratio.
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Table of contents

Abstract ............................................................................................................................................. i
Acknowledgements .......................................................................................................................... ii
Table of contents ............................................................................................................................... iv
Table of figures .................................................................................................................................. vi
Abbreviations .................................................................................................................................... viii
1. Introduction ...................................................................................................................................... 1
   1.1. Main goal .................................................................................................................................. 3
2. Materials and methods .................................................................................................................. 4
   2.1. Polyvinyl-Alcohol Microbubbles .............................................................................................. 4
   2.2. Experimental setup .................................................................................................................. 5
   2.3. Contrast-to-Tissue and Contrast-to-Noise Ratios ................................................................... 8
   2.4. Ultrasound Imaging Techniques .............................................................................................. 10
3. Results ............................................................................................................................................ 13
4. Discussion ....................................................................................................................................... 17
   4.1. Limitations ............................................................................................................................... 19
   4.2. Future work ............................................................................................................................... 20
5. Conclusion ...................................................................................................................................... 21
References .......................................................................................................................................... 23
Appendix ............................................................................................................................................ 26
   A.1. Introduction ............................................................................................................................. 26
   A.2. Contrast-Enhanced Ultrasound ............................................................................................... 27
   A.3. Microbubbles ............................................................................................................................ 28
      A.3.1. Evolution of Microbubbles ............................................................................................... 28
      A.3.2. Drawbacks of MBs ............................................................................................................. 30
   A.4. Microbubble behavior at different mechanical indexes .......................................................... 32
A.5. Methods of detection of microbubbles for imaging ........................................ 34
A.5.1. Early imaging techniques ........................................................................ 35
A.5.2. Non-Destructive Imaging Techniques ...................................................... 36
A.5.3. Destructive Imaging Techniques ............................................................. 39
A.5.4. Ultraharmonic and Subharmonic Imaging ............................................... 40
References ........................................................................................................ 43
Table of figures

Figure 1: Experimental setup. ........................................................................................................6
Figure 2: Figures shown after running a script: (a) GUI, (b) RF Data, (c) Amplitude Spectrum, (d) Real time Display. ........................................................................................................7
Figure 3: Example of the 3 ROIs on the gray-scale image for the specific transducer position: MBs (left-red rectangular), Tissue (center-green rect.), Water (blue-right rect.). 9
Figure 4: Equiripple FIR 11-tap filters around 3 (left) and 9 (right) MHz. .................................12
Figure 5: Gray scale images obtained with each of the six techniques. .................................14
Figure 6: Amplitude Spectra of the RF Data from MBs, tissue-mimicking phantom and water obtained with each of the six techniques.................................................................15
Figure 7: Schematic illustration of the bubble behavior at different MI regions [15]...........33
Figure 8: Schematic illustration of PI technique. (a) First Pulse, (b) Second pulse with inverted polarity, (c) Summation of the two pulses results in elimination of the linear components, leaving only the nonlinear echo [20] .................................................................39
Figure 9: Spectra of scattered signals from SonoVue microbubbles, insonified at frequency 4 MHz [22]...........................................................................................................................................41
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>CEUS</td>
<td>Contrast-Enhanced Ultrasound</td>
</tr>
<tr>
<td>MB</td>
<td>Microbubble</td>
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<tr>
<td>CT</td>
<td>Computed Tomography</td>
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<tr>
<td>TI</td>
<td>Thermal Index</td>
</tr>
<tr>
<td>MI</td>
<td>Mechanical Index</td>
</tr>
<tr>
<td>Pr</td>
<td>Peak rarefraction pressure, measured in Pa</td>
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<tr>
<td>CFI</td>
<td>Color Flow Imaging</td>
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<tr>
<td>PDI</td>
<td>Power Doppler Imaging</td>
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<td>CHI</td>
<td>Contrast Harmonic Imaging</td>
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<tr>
<td>PI</td>
<td>Pulse Inversion</td>
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<tr>
<td>CPS</td>
<td>Contrast Pulse Sequence</td>
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<tr>
<td>CNR</td>
<td>Contrast-to-Noise ratio</td>
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<tr>
<td>CTR</td>
<td>Contrast-to-Tissue ratio</td>
</tr>
<tr>
<td>Rpm</td>
<td>Rounds per minute</td>
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<tr>
<td>ROI</td>
<td>Region of Interest</td>
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<td>BF</td>
<td>Bandpass Filter</td>
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1. Introduction

The echoes produced by the small vessels with the conventional ultrasound are often found to be insufficient, comparing to the surrounding tissue. The anatomy of the heart and the complicated cardiovascular system introduce further difficulties along the way [1,2]. In addition, the use of procedures that utilize radioactive materials rise the concern that the accumulative dose received by patients may create new problems. On the contrary, ultrasound is an imaging technique that does not utilize radioactivity, fact which makes it extremely useful for diagnostic purposes. Hence, research has been put onto developing new efficient methods for imaging small vascular structures in the body with ultrasound.

The confidence in the diagnosis has been found to increase by introducing the Ultrasound Contrast Agents (UCAs), which are basically developed to improve the echo from blood. Starting with generation 0 and the form of free gas bubbles, the UCAs have, nowadays, reached generation 3 and evolved to ‘particulate’ (e.g. polymer shell) gas bubbles with controlled acoustic properties [1,2]. With the introduction of the most widely used agents known as microbubbles (MBs), the weak echoes produced by the small vessels, which are in general difficult to detect, can be enhanced and, together with new methods of Ultrasound Imaging, could make possible the identification of the small vascular structures in the body, such as the small myocardial vessels [1].

When exposed to ultrasound, MBs begin to oscillate. Depending on the acoustic pressure they are exposed to, the MBs have three regimes, in which their behavior of oscillation changes: the low-, the intermediate and the high-pressure or destructive regime. Of particular interest is the way they oscillate in the non-destructive regime. This oscillation generates components at the frequency they are exposed to (fundamental), but also at different frequencies. Especially the emissions of components at double the fundamental frequency (second harmonic) from the MBs have been an area of extensive research. However, according to previous research [3-5], it has been found that there is a threshold pressure, above which sub- and ultraharmonics can also be generated. Focusing at these regions is found to enhance Contrast-to-Tissue (CTR) and Contrast-to-Noise (CNR) ratios of the imaging techniques in respect to focusing on the fundamental or second harmonics, since the sub- and ultraharmonic components are mainly generated by the MBs and not
by the tissue. The imaging performance is found to further increase, when the Pulse Inversion (PI) Imaging technique is included, in an effort to suppress the response from the linear tissue (See Appendix).

The feasibility of combining the behavior of the MBs at the sub- and ultraharmonic regions has not yet been researched extensively. Previous trial of combining the two modes of Sub- and Ultraharmonic Imaging by Shekhar et al., has reported a large improvement of the CTR value [13]. Thus, the feasibility of combining these two imaging techniques together in a Contrast Pulse Sequence (CPS), including the PI technique, would be worth investigating for a different setup and type of MBs.
1.1. Main goal

The main goal of this master thesis can be divided into two parts: the first part was to create the setup and implement ultrasound imaging techniques on the Verasonics Research System® [6,7], in order to exploit the different behavior between the tissue and the Polyvinyl-Alcohol (PVA) MBs in the ultraharmonic region, at the non-destructive regime. The second part was to assess the response of the PVA MBs in the ultraharmonic region, while evaluating the imaging performance of the implemented ultrasound imaging modalities.
2. Materials and methods

This section provides a description of the materials used for the experiment, as well as the ultrasound imaging techniques that were implemented. Summary of the PVA MBs, the tools and equipment used in the lab to obtain the results and parameters manipulated in order to develop the algorithms of the ultrasound imaging techniques on the Verasonics Research System® are mentioned, among others.

2.1. Polyvinyl-Alcohol Microbubbles

The suspension with the MBs was fabricated based on a previously developed protocol [8]. In short, the procedure had two phases, the suspension preparation and the washing. During the first step, PVA powder was mixed with Milli-Q water in a beaker under high agitation. The next step was to add NaIO₄ into the mixture, for the creation of plain MBs. A separation funnel was used for the washing part of the procedure.

After the fabrication, size and concentration of the bubbles were measured by using a microscope (ECLIPSE Ci-S, Nikon, Tokyo, Japan), according to the procedure described in [9]. The concentration was measured to be 8.8x10⁷ MBs/mL.

For these bubbles, the resonant frequency has been reported, according to previous studies, to be within the range 7-10 MHz [10,11].
2.2. Experimental setup

The experimental setup consisted of the following components and can be seen in Figure 1:

1. Host Computer with installed MATLAB (R2013a, MathWorks, Natick, USA),
2. Verasonics Research System® (Verasonics Inc., Kirkland WA, USA),
3. L7-4 ultrasound linear array transducer (Philips Healthcare, Andover, MA, USA),
4. Tissue-mimicking phantom (Model 524 Peripheral Vascular Doppler Flow Phantom, ATS Laboratories),
5. Peristaltic pump (Watson Marlow Peristaltic Pump sci 323),
6. Magnetic stirrer (IKA REO Basic C Laboratory Magnetic Stirrer, Asus Technologies Ltd),
7. Container with the PVA MBs solution.

For the generation of the waves, the L7-4 linear array transducer was connected to the Verasonics Research System® [6,7], which was connected to the host computer. Implementation of the ultrasound imaging techniques was performed by developing Contrast Pulse Sequences (CPS) using MATLAB on the host computer. The transducer was placed above the phantom, perpendicular to the tubes, in a way to image two of them: the 6mm tube containing the solution with the PVA MBs and the 8mm tube containing degassed water. The peristaltic pump was used to control the flow of the solution from the container to the 6mm tube of the phantom. The pump was turned on to insert the solution with the MBs into the tissue-mimicking phantom, in the 6mm tube. After 5 seconds, the pump was turned off and at the same time ultrasound pulses began to transmit in the phantom, for 2 seconds, for one measurement to take place. The same procedure was performed ten times for each of the techniques. Dilution of the MBs was done in a container with degassed water, in order to reach the concentration of $10^6$ MBs/mL. The container with the solution was placed on the magnetic stirrer, which was stirring the solution at 100 rounds per minute (rpm), so that MBs were equally dispersed in the container. Two plastic tubes were used to unite the start and end of the 6mm tube of the phantom, creating a closed circuit. Above the phantom, transmission gel (Ultrasound Transmission Gel AQUASONIC 100) was placed between the surface of the phantom and the transducer, in order to create a tight bond between the phantom and the transducer.
Example scripts developed for MATLAB provided by Verasonics were manipulated for introducing the ultrasound imaging techniques [6,7]. Additional scripts were developed for creating the amplitude spectra from the RF Data of the Regions of Interest (ROIs) in real time and calculating the CTR and CNR ratios from the gray-scale images. The four figures shown after running the scripts were (Figure 2):

a) Graphical User Interface (GUI) enabling the user to change the following parameters: Time-Gain-Compensation (TGC), speed of sound in tissue, sensitivity, digital gain, voltage output, channel (element of the transducer) of interest (Plot), depth of focus (TX), compression, reject and persistence levels,
b) RF data for the channel of selection,
c) Amplitude Spectrum of a particular ROI (red area in RF Data figure) of the same channel,
d) Live display of the tissue-mimicking phantom in gray-scale mapping.
Figure 2: Figures shown after running a script: (a) GUI, (b) RF Data, (c) Amplitude Spectrum, (d) Real time Display.
2.3. Contrast-to-Tissue and Contrast-to-Noise Ratios

The performance of each technique was determined by calculating the parameter CTR from the gray-scale image, following the equation [12]:

\[
CTR(dB) = 20 \log_{10} \frac{\text{mean}(A)}{\text{mean}(B)} \tag{1}
\]

\[
CNR(dB) = 20 \log_{10} \frac{\text{mean}(A)}{\text{mean}(C)} \tag{2}
\]

Where,
Mean(A) is the mean pixel value from the ROI containing the MBs (6mm tube),
Mean(B) is the mean pixel value from a ROI in the tissue-mimicking phantom,
Mean(C) is the mean pixel value from the ROI containing degassed water (8mm tube).

An example of the three ROIs used can be seen in Figure 3. Each ROI was selected according to the following parameters: width = 85 pixels, height = 60 pixels, area = 5100 pixels. The transducer was placed above the tissue-mimicking phantom in a vertical position above the two tubes of interest, the 6mm tube containing the MBs and the 8mm tube filled with water. In Figure 3, the position of the transducer can be seen, as well as an example of the gray scale image acquired with this setup, together with the selected ROIs. The selected ROIs were selected to have rectangular shapes, inside the 6mm, 8mm tubes and in-between them at the same depth, as can be seen in Figure 3. In Figure 3, the 6mm tube can be seen in the left of the gray scale image and the 8mm tube can be seen in the right. The rectangular ROI for the MBs was selected in the 6mm tube and was colored red, the ROI for the water was selected in the 8mm tube and was colored blue, while the ROI for the tissue was selected between them and was colored green. The x-axis of the gray-scale image represented the different channels (elements) of the L7-4 transducer (128 elements, 38mm Field of view), while the y-axis represented the depth inside the phantom in terms of wavelength: e.g. 10 meant 10 times the current wavelength, while the speed of sound in tissue-mimicking phantom was 1450 m/s. Hence, the dimensions of the gray scale images were 38mm(width) and 21.75mm(height). It is worth mentioning here that since the Verasonics system used by default a square root compression function in the processing of the pixel values for the display window, the pixel...
values were squared, before calculating the mean pixel values from the image and eventually the CTR (persistence processing should be turned off, pgain set equal to 1.0 and compression to 0.5) [5].

Figure 3: Example of the 3 ROIs on the gray-scale image for the specific transducer position: MBs (left-red rectangular), Tissue (center-green rect.), Water (blue-right rect.).
2.4. Ultrasound Imaging Techniques

The ultrasound imaging techniques implemented in this project are described in this section. It needs to be stated here, that the reconstruction of the gray scale images by the RF data was performed entirely by the Verasonics system in each and every case. The user had limited access and ability to manipulate the reconstruction process, hence the contribution of the user in the reconstruction process is minimal. The differences between the techniques concern manipulation of the RF data acquired and not the reconstructed images directly.

2.4.1. Fundamental B-Mode

For reference purposes, the Fundamental B-Mode or Conventional B-Mode imaging technique was implemented. Single pulses were transmitted at 6 MHz, the echoes from the phantom were received without the additional design of any filtering by the user, and the image was reconstructed by the Verasonics system from the RF data.

2.4.2. Ultraharmonic

For the isolation of the ultraharmonic components, an Equiripple FIR 11-tap Bandpass Filter (BF) around 9 MHz (ultraharmonic) was implemented, in agreement with the requirements of the Verasonics system. The parameters and the response of the filter can be seen in Figure 4. Single pulses were transmitted at 6 MHz, the echoes from the phantom were received with the implementation of the aforementioned filter by the user, and the image was reconstructed.

2.4.3. Pulse Inversion

For maximization of the MBs detection performance, the Pulse Inversion (PI) technique was implemented [12]. In this case, two pulses with opposite polarity were transmitted at 6 MHz in sequence with a short time period between them for each beam line, sufficient
enough to let the transducer transmit each pulse and detect the echoes separately (Appendix). These echoes were then summed together to obtain one beam. After the summation of the echoes, the ones generated by the tissue should ideally be mutually cancelled (fundamental frequency region), leaving only the echoes produced by the nonlinear behavior of the PVA MBs. No filtering was performed in this case. The reconstruction process was performed once again by the Verasonics system from the summed RF data.

2.4.4. Ultraharmonic Pulse Inversion

Similar to the Pulse Inversion technique (2.4.3), the Ultraharmonic Pulse Inversion was selected in order to take advantage of both the tissue suppression that the PI provided, as well as the nonlinear behavior of the PVA MBs around the ultraharmonic region. Two pulses were transmitted once again with opposite polarity at 6 MHz in sequence, with the only difference the addition of the Equiripple BF for the isolation of the ultraharmonic components (Figure 4). The filtered RF data were summed, and the image was reconstructed.

2.4.5. Subharmonic Pulse Inversion

This technique was implemented mainly because of the final Contrast Pulse Sequence (2.4.6) and was kept for reference purposes. The idea here was similar to the Ultraharmonic Pulse Inversion. Two pulses were transmitted with opposite polarity at 6 MHz, but the Equiripple BF was designed for the isolation of the subharmonic region in this case (3 MHz). The filter response is seen in Figure 4. The filtered RF data were summed together and the image was reconstructed by the Verasonics System.

2.4.6. Contrast Pulse Sequence

The idea behind this experiment was that both subharmonic and ultraharmonic response were generated mostly from the bubbles and not the linear tissue [4,5]. Previous trial of
combining the two modes by Shekhar et al., reported an improvement of the CTR value [13]. Thus, in order to further improve the imaging performance of our setup, the feasibility of combining these techniques together in one CPS including the PI technique was investigated, in an effort to minimize the response from the tissue around the fundamental frequency. The CPS consisted of 4 pulses transmitted in sequence: two pulses were transmitted with opposite polarities at 6 MHz and filtered using the aforementioned Equiripple BF around 9 MHz, and 2 pulses were transmitted at 6 MHz but this time the filtering was performed around 3 MHz (subharmonic region), one again with inverted polarity. The response and parameters of both filters can be seen in Figure 4.

For all the techniques, the transmitting frequency of the pulses was selected at $f_0=6.0$ MHz, while each pulse consisted of 1 cycle. Peak-to-peak voltage was selected at 60V, in order to force the bubbles to oscillate in a nonlinear fashion, but at the same time remain at the non-destructive regime. Time-Gain-Compensation was added in order to enhance the echoes of the ROI, the same for all measurements. The 128-element L7-4 linear transducer enabled the implementation of dynamic focusing, by using time delays between the transmission of each pulse for each element. The focus was selected to be at the depth of the center of the 6mm tube inside the phantom, at 60 wavelengths. The CTR and CNR value were calculated from 10 gray-scale images, and the average over the 10 frames were estimated for each and every case, in order to produce the final results.

Figure 4: Equiripple FIR 11-tap filters around 3 (left) and 9 (right) MHz.
3. Results

In this section, CTR and CNR values are reported for every different technique, while the amplitude spectra and gray-scale images are presented. Figure 5 shows the acquired gray-scale images for each of the 6 implemented ultrasound imaging techniques described in section 2.4. In each of these images, the contrast between the 6mm tube, the 8mm tube and the in-between tissue-mimicking phantom was calculated and averaged over 10 frames. The results are seen in Table 1.

Figure 6 shows the obtained amplitude spectra from the RF Data of the ROI of the MBs in red, tissue-mimicking phantom in green and water in blue, for each different technique. For the final amplitude spectra, 10 amplitude spectra (one for each frame), were acquired and averaged, for each of the 6 techniques, for each ROI. The channel of interest was manipulated according to the ROI: for the MBs the channel of interest was selected at 25, for the water the channel of interest was selected at 105, while for the tissue the channel of interest was selected at 65.

Table 1 summarizes the CTR and CNR values for each of the six imaging techniques.

<table>
<thead>
<tr>
<th>Imaging techniques/Ratios</th>
<th>CTR (dB)</th>
<th>CNR (dB)</th>
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<tr>
<td>Fundamental B-Mode</td>
<td>0.06</td>
<td>23.01</td>
</tr>
<tr>
<td>Ultraharmonic</td>
<td>0.06</td>
<td>20.53</td>
</tr>
<tr>
<td>PI</td>
<td>9.78</td>
<td>36.75</td>
</tr>
<tr>
<td>Subharmonic PI</td>
<td>5.81</td>
<td>33.89</td>
</tr>
<tr>
<td>Ultraharmonic PI</td>
<td>8.83</td>
<td>32.82</td>
</tr>
<tr>
<td>CPS</td>
<td>13.27</td>
<td>37.55</td>
</tr>
</tbody>
</table>

Table 1: Synopsis of the CTR and CNR values for each of the six imaging techniques.
Figure 5: Gray scale images obtained with each of the six techniques.
Figure 6: Amplitude Spectra of the RF Data from MBs, tissue-mimicking phantom and water obtained with each of the six techniques.
The comparison shows that the Fundamental B-Mode imaging technique reports both the lowest CTR at 0.06 dB and the second lowest CNR at 23.01 dB. Similar is the CTR in the case of the Ultraharmonic imaging technique, while a lower CNR can be observed, a fact showing that although the Equiripple BF suppressed the tissue response mainly around the fundamental frequency, it also suppressed the response from the MBs at this region. This can be also seen in Figure 6 showing the amplitude spectra for the Fundamental and the Ultraharmonic imaging techniques. The response at both the central frequency (6 MHz) and 1.5 harmonic decreases with the implementation of the filter, while no particular peek can be seen around the ultraharmonic region.

On the other hand, significant is the difference in ratios when introducing the PI technique. PI reports an improvement of almost 10 dB from the Fundamental B-Mode in CTR and even higher in CNR. A slight decrease in CNR can be noticed when combining the PI technique with the Equiripple BF. For the Subharmonic PI technique, there is also a significant decrease in the CTR value of approximately 4 dB, while for the Ultraharmonic PI the CTR remains almost at the same level as the PI. From the amplitude spectra (Figure 6), the suppression of the tissue response becomes clear, when implementing the PI technique, almost 30 dBmV between the Fundamental B-mode and the PI technique, around at the same level between the Ultraharmonic and the Ultraharmonic PI.

Finally, an increase both in CTR of MBs and in CNR can be noticed with the combination of the Sub- and Ultraharmonic PI techniques, by using a CPS consisted of four pulses. This technique reported the highest values both in CTR and CNR. Also, significant peeks can be seen in the respected amplitude spectra, although the simultaneous implementation of the filters for the isolation of the sub- and ultraharmonic regions and the PI technique are the main factors for these peeks.
4. Discussion

Results show that for the PVA MBs, the Equiripple BF used did not provide significant changes in terms of CTR and CNR (only 2 dB increase between the ‘no-filter’ techniques), although large differences can be seen in the amplitude spectra. This could be explained by two factors. First, although the use of the filter for isolation of the sub- and ultraharmonic components had been thoroughly investigated and Equiripple BF were found to be one of the most suitable (among the filters that could be implemented by the Verasonics system) [17], yet, the introduction of the Equiripple BF for the isolation of the sub- and ultraharmonic regions also affected the bubble response around the fundamental frequency.

Second, it should be noted that from all the amplitude spectra acquired for the different ultrasound imaging techniques (Figure 6), the response of the particular type of PVA MBs at both the sub- and ultraharmonic regions was rather small, even though enough voltage was used (60 volts peak-to-peak), to ensure the generation of nonlinear oscillations, but at the same time remain at non-destructive region. The reason is that according to previous research [11], although these specific bubbles do indeed generate second, sub- and ultraharmonics, they do so at high-pressure amplitude excitations (e.g. Pr = 1 MPa). In that region (High MI), the bubble shell fractures and gas is squeezed out through the cracks. It is this “pumping-out” behavior that is characterized by the presence of higher and lower harmonics. Therefore, it is possible that the pressure amplitude used for excitation in this study was not sufficient enough to ‘break’ the shell of the bubbles, resulting in higher response from the sub- and ultraharmonic regions.

On the other hand, the use of the PI technique increased considerably the CTR and CNR values. The reason is that it achieved a substantial suppression of the linear tissue response, while its effect at the sub- and ultraharmonic regions was not that significant.

Finally, the combination of the Sub- and Ultraharmonic PI Imaging techniques into one CPS consisted of four pulses reported some rather counter-intuitive results. It provided higher CTR than all the techniques (around 4 dB higher than the next highest) and also slightly higher CNR. This fact is also depicted in Figure 5, where the visualization of the tube containing the MBs is improved with CPS, compared to the other techniques. It has
to be mentioned that the combined technique improves the CTR by approximately 5 dB than the Ultraharmonic PI and by 8 dB than the Subharmonic PI mode. The almost 14 dB of CTR value it provided can be characterized efficient for imaging [14,15], in contrast with the other techniques, for which the CTR value did not exceed 10 dB.

The results described in this report can be compared with the results in [14]. In [14], a different way of combining the sub- and ultraharmonic modes was described. An improvement of around 15 dB was reported, between the combined mode and the single modes. However, in that case phospholipid-shelled UCAs were visualized at 12 MHz and 30 MHz fundamental frequencies, while the CTR and CNR were calculated by using the average power of the corresponding ROIs, as it is explained in [16].

In [15], similar poly (vinyl alcohol)-shelled microbubbles were used, and six different techniques were implemented for imaging. The performance was measured by calculating the CTR values. The CTR values acquired with CW pulses were close to 0 dB, as in our Fundamental B-Mode imaging. Implementation of the PI reported an increase of about 5-7 dB, while in our case the rise was almost 10 dB. The chirp CPS3 reported the highest CTR value in that experiment, reaching almost 25 dB.
4.1. Limitations

A number of factors limited the results of this project. First of all, the results in this study were obtained using one particular ultrasound transducer, the linear array L7-4. This transducer’s sensitivity is highest in the region 4-7 MHz and lower outside. This fact limited the acquired response from the bubbles at the sub- and ultraharmonic regions, which were outside the bandwidth of the transducer (at 3 MHz and at 9 MHz respectively).

Moreover, given the limited frequency bandwidth of the transducer and the fact that the resonant frequency of the MBs is directly related to their sizes, our system was optimized for only a small portion of the entire polydisperse population of PVA MBs [18].

Third, the Verasonics system that was used for the manipulation of the transmitting parameters, acquisition of the RF Data and reconstruction of the image limited our choices regarding the transmitting pulse waveform, the transducer and the filters that were implemented for the isolation of the sub- and ultraharmonic regions in the cases of: a) Harmonic Imaging, b) Harmonic PI, c) Subharmonic PI and d) CPS. As a result, manual experiments were performed in an effort to generate the most efficient filter for our current setup, from the available ones. The Equiripple BF was selected as the most suitable for the case. However, even with this filter, the isolation of the sub- and ultraharmonic regions can by no means be considered perfect.

Finally, as it has already been stated, using higher-amplitude for excitation (higher peak-to-peak voltage), would generate higher response at the sub- and ultraharmonic regions. However, the case in this study was the characterization of the bubble response at the non-destructive regime.
4.2. Future work

Future work should focus on optimizing the parameters on the Verasonics system, for our current experimental setup, for instance implementing the same ultrasound imaging techniques for a transducer with a broader bandwidth. Such a transducer (e.g. L12-5) would not limit the acquired response at the sub- and ultraharmonic regions, thus receiving echoes of higher amplitude from these regions, where only MBs could generate.

In addition, using a monodisperse suspension of MBs could enhance the response at the sub- and ultraharmonic regions. The reason is that if all bubbles had the same size, then they all would resonate at the same frequency. By manipulating the system accordingly, sharper peaks could be generated at the regions of interest on the frequency spectrum.

Furthermore, research should be put on optimizing the waveform of the transmitted pulse (e.g. number of cycles, chirp), as well as the filter for the isolation of the sub- and ultraharmonic regions. A more efficient filter would ideally suppress even further the response from the tissue, leading to an increase in CTR and CNR values. However, it needs to be stated here that the Verasonics system limits the choice regarding the filter and the transmitted pulse. Thus, a way in order to bypass these limitations needs to be investigated.

Last but not least, for further evaluation of the efficiency of the ultrasound imaging techniques, it would be beneficial to use a different type of MBs, ideally ones with proven ability to generate ultraharmonic components at the non-destructive regime and use it as reference for further studies.
5. Conclusion

In this study, a setup was created for the implementation of ultrasound imaging techniques on the Verasonics Research System®. Six ultrasound imaging techniques were implemented, as a way to take advantage of the different behavior between the tissue (mimicking phantom) and the PVA MBs in the ultraharmonic region, with focus at the non-destructive regime.

The detection of the generated ultraharmonic components from the PVA MBs at the non-destructive regime was limited with our current setup. A different setup could be used for further evaluation of their response.

In addition, it was demonstrated that the combination of the Subharmonic and Ultraharmonic PI techniques increased the imaging performance, in comparison with the separate implementation of them. By using a different setup or a different type of MBs, one could investigate further the feasibility of combining the Subharmonic and Ultraharmonic PI imaging techniques into one CPS.
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Appendix

A.1. Introduction

Since 1880 and the discovery of the piezoelectric effect by Curie brothers, ultrasound has travelled a long way until today [1]. Nowadays, ultrasound is used in excess for a wide variety of clinical purposes, with applications in both therapeutic and imaging procedures. Ultrasounds are pressure waves on frequencies that are higher than the human hearing range. The frequency a clinical ultrasound application typically uses fits in the range between 1-15 MHz.

Ultrasound has been established as one of the most widely used imaging modalities for diagnostic purposes, due to the several advantages it provides in comparison with other techniques (e.g. magnetic resonance imaging or computed tomography). Its capability to image non-invasively, in real time with high temporal and spatial resolution (mainly on high frequencies), as well as its low cost has drawn the attention of the scientific community. In addition, the mobility and portability it provides makes it a practical tool in the hands of the doctors and medical staff. The greatest disadvantage of ultrasound imaging is the need for highly experienced and well-educated operators, in order to achieve qualitative images and accurate diagnoses [2].

The confidence in the diagnosis provided by an ultrasound examination can now further increased by the use the Contrast-Enhanced Ultrasound (CEUS) technique.
A.2. Contrast-Enhanced Ultrasound

The application of CEUS is not new. In fact, agents for improving medical ultrasound imaging have been proposed since 1968, although organized research did not begin until approximately 10 years later. The safety this technique provided both for adults and children, in comparison with the radiocontrast agents or the agents used for CT scans, made it particularly appealing for use in medical diagnosis [3]. The cornerstone, on which the idea of CEUS is based, is the fact that the interfaces between different substances cause the sound waves to be reflected or scattered in various ways.

Ultrasound contrast agents (UCAs) are developed in an effort to increase the reflection of sound, providing greater backscattering. This results in images of increased contrast, due to difference between their level of echogenicity in comparison with the surrounding tissue [4]. This fact becomes crucial in the case of investigating the blood flow in small vessels or the volume and movements of cavities filled with blood. Especially in the case of myocardium, the sophisticated anatomy of the heart and the complicated cardiovascular system generate obstacles during the estimation and imaging of the myocardial perfusion and volume [3,5]. With the introduction of UCAs, the weak echoes produced by the small myocardial vessels, which are in general difficult to detect, can be enhanced and together with new methods of Ultrasound (e.g. harmonic/pulse inversion imaging) could improve the assessment of the myocardial perfusion, in addition to a clearer identification of the border of the endocardium [5]. The most widely used agents for this modality are known as microbubbles (MBs).
A.3. Microbubbles

MBs are simply defined as exogenous substances, which are injected (usually) in the body either in a cavity or a blood pool, in order to improve the signals obtained by the ultrasound technique [6,7]. The material used for fabricating them is not limited to only one, but it can rather take many forms such as: solid particles in suspension, gas bubbles, encapsulated gases or liquids, or aqueous solutions [7]. Administration of MBs and observation of them can enhance the diagnostic power and sensitivity of ultrasound imaging significantly [6]. In order to be effective during ultrasound imaging, MBs require to have the following properties: non-toxicity, ability to be injected intravenously either by bolus or infusion, stability during various passages within the body, and, finally, ability remain within the blood pool for adequate time during the examination, before metabolized or removed safely from circulation afterwards [5,7].

A.3.1. Evolution of Microbubbles

• Generation 0
The very first injected ultrasound contrast agent is known to be the free gas bubbles that were used by Gramiak and Shah in 1968, in a way to improve the echo received from blood pool. More specifically, during an echocardiographic recording, they injected a solution into the ascending aorta and which they observed a “cloud” of echoes from the aorta and the chambers of the heart. The bubbles were created either during the injection (cavitation) or by stirring of the saline (agitation). The intensity of the produced echoes mainly depends on the type of the solution, but they are generally good scatterers of sound energy. However, the fact that they are filtered by the lungs due to their size, as well as their instability, makes them impractical for certain types of imaging [5,7].

• Generation 1
As a way to improve the stability of the injected bubbles, the idea of encapsulating the bubbles within a shell arose. Carroll et al. in 1980 were the first to manufacture encapsulated air bubbles, consisted of nitrogen gas and a gelatin shell. They injected them into the femoral vein of rabbits and reported ultrasound enhancement of the tumor rims
However, the size still remained a serious issue. Attempt to resolve the problem was first made by Feinstein four years later, who successfully managed to fabricate MBs by applying sound energy to agitate particles in a solution of human serum albumin (sonication) [5,7,9].

- **Generation 2**
  For further enhancement of the stability and the backscattering, new agents were designed, eventually named as low solubility gas bubbles. The main difference between these bubbles and the ones of the previous generation, is the fact that the newer are fabricated using with low solubility gases, which reduce the rate of diffusion from the thin shells, thus increasing the time available for imaging [5].

- **Generation 3**
  As a way to not only improve the stability and backscattering, but also controlling the acoustic properties, a new generation made its appearance: the ‘particulate’ MBs. These bubbles are fabricated by using a variety of different materials for shells and gases. The shell material generally determines the longevity of the bubbles in the body. The elasticity of it also regulates the amount of acoustic energy a bubble can withstand, before the shell brakes (burst). Currently, the material used for MB shells consists usually of galactose, lipid, albumin or polymers. On the other hand, the gas encapsulated determines the echogenicity of the bubble. Heavy gases tend to dissolve less in water, which enables the MBs containing them last for a longer period of time, compared to air bubbles. In Table 1, some currently produced ultrasound contrast agents for commercial use are mentioned [4,10].

<table>
<thead>
<tr>
<th>Name</th>
<th>Manufacturer</th>
<th>Shell</th>
<th>Gas</th>
<th>Mean diameter (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albunex</td>
<td>Molecular Biosystems</td>
<td>Albumin Air</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>Optison</td>
<td>Mallinckrodt/Amersham</td>
<td>Albumin Octafluoropropane</td>
<td>2–4.5</td>
<td></td>
</tr>
<tr>
<td>Sonovue</td>
<td>Bracco Diagnostics</td>
<td>Lipid Sulphur</td>
<td>2–3</td>
<td></td>
</tr>
<tr>
<td>Levovist</td>
<td>Schering AG</td>
<td>Lipid/galactose</td>
<td>Air</td>
<td>2–4</td>
</tr>
</tbody>
</table>

*Table 2: Commercially available Ultrasound contrast agents [10].*
A.3.2. Drawbacks of MBs

Despite all the advantages CEUS and MBs offer in comparison with the other imaging modalities, there are certain weaknesses that are still under research. First of all, as it has been mentioned, MBs do not last in circulation for a big amount of time, which makes the real-time imaging a challenge. Furthermore, absorption of the ultrasound waves by the tissue can cause increase in temperature. This phenomenon is usually measured with the thermal index (TI):

\[
TI = \frac{W_p}{W_{\text{deg}}}
\]

Where,
Wp: relevant (attenuated) acoustic power at the depth of interest,
Wdeg: estimated power necessary to raise the tissue equilibrium temperature by one degree Celsius [11].

High TI may result in hazard, especially in applications of obstetric, neonatal and adult transcranial scanning [12,13].

Finally, an important factor that needs to be addressed is the mechanical index (MI). MI describes the possibility of a mechanical (non-thermal) damage in a homogeneous tissue and is defined as:

\[
MI = \frac{Pr}{\sqrt{f}}
\]

Where,
Pr: the peak rarefaction (negative) pressure in situ,
f: the ultrasound frequency.
In general, high MI increases the quality of the image, but the risk of cavitation increases with contrast agents. Behavior of the MBs in respect to different levels of MI will be explained in more detail in the next subchapter.

A popular way to assure safe use of diagnostic ultrasound is the ALARA principle, abbreviation for ‘as low as reasonably achievable’, meaning that an examination should be performed for the shortest time and lowest power possible, in order to acquire the needed diagnostic information [4,14].
A.4. Microbubble behavior at different mechanical indexes

When a microbubble is exposed to an ultrasound wave, it begins to oscillate. During the positive part of the cycle (compression) it contracts, while during the negative part (rarefraction) it expands.

The frequency at which the oscillation of a bubble is maximum is called resonant frequency and it depends mainly on its size (diameter) and also on the type of shell.

MBs oscillate in a different way, depending on the acoustic pressure of the incident sound wave. There are three regimes that determine the way of oscillation of a MB (Figure 1):

- **Low MI region**
  This regime is characterized by low acoustic pressure, typically below 100 kPa, resulting in an MI of under 0.1, at 1 MHz. In this regime, the MBs undergo a linear oscillation, producing linear backscattered enhancement. The incident sound wave and the scattered signal are on the same frequency [5,12].

- **Intermediate region**
  As the acoustic pressure rises above 100 kPa, resulting in an increase of the MI above 0.1 (at 1 MHz), the gas in the MBs force them to contract less than they expand. Thus, their oscillation is nonlinear in this region. Due to this nonlinear behavior, the scattered signals from the bubbles differ from the incident in magnitude and frequency. This makes certain frequencies appear eventually in the scattered wave, other than the fundamental one, called harmonics. This harmonic backscatter is the foundation of the CEUS imaging modes with MBs, such as the pulse inversion. The frequency two times the fundamental one is called second harmonic, three times the fundamental is called third harmonic, while the frequency half the fundamental is called subharmonic [5,12].

- **High MI region**
  After the acoustic pressure reaches a high level, typically above 1 MPa, resulting in MIs above 1 (at 1 MHz), the MBs start to burst and the gas inside them is released. This disruption forms transient harmonic echoes, resulting in a scattered wave of a wide-frequency range, which strongly scatters the ultrasound. However, due to fast dissolving of the collapsed bubbles in bloodstream, timing of imaging or estimation is crucial [5,12].
Here, it needs to be stated that the boundaries between the different regimes and oscillation behaviors of the MBs are not exactly sharp and they differ from one contrast agent to another, depending on the material used for the gas and the shell. Also, acoustic pressure is not the same in every direction of the scan plane, resulting in locally different MI, causing often varied interactions between the MBs [5,12]. For the purpose of this project, the Low and Intermediate MI regimes are together mentioned as the Non-Destructive regime, while the High-MI regime is considered to be the Destructive regime.

Figure 7: Schematic illustration of the bubble behavior at different MI regions [15].
A.5. Methods of detection of microbubbles for imaging

In this chapter, a short summary of the methods used for the detection of MBs for imaging will be presented. A more detailed analysis of the methods can be found in [16]. For purposes of simplicity, the techniques used for visualization of the MBs will be divided into three main categories, while the fourth category introduces the reader to the concept of the sub- and ultraharmonic imaging:

- Early imaging techniques,
- Non-destructive imaging techniques,
- Destructive imaging techniques,
- Sub- and Ultraharmonic imaging techniques.

Considering the scope of this master thesis, emphasis is put onto explaining and comparing the early and non-destructive techniques, while the destructive are only shortly mentioned.

Before proceeding to the summary of the existing methods for imaging MBs, some qualities of interest will be introduced, in order to ensure a better understanding of the differences between the techniques.

**Sensitivity:** Proportion of the correctly identified positives by the probe elements (true positive rate) [17].

**Contrast Resolution:** Measures the quality of acquired images, defined as the ability to distinguish differences in intensity in an image [18].

**Spatial Resolution:** Ability of the system to distinguish two points as separate in space [19]. It is divided into axial and lateral resolution. **Axial resolution** is the resolution in the direction parallel to the ultrasound beam, while **lateral** is the resolution perpendicular to the ultrasound beam.

**Temporal resolution:** the ability of the system to detect that an object has moved over time. For the purposes of medical ultrasound, temporal resolution is synonymous with frame rate [19].
A.5.1. Early imaging techniques

These techniques were the first techniques developed for visualizing MB-based agents. They simply make use of the fact that the power of scattering by a microbubble is much larger than that of a blood cell. Even without using advanced methods or equipment, MBs can increase the backscattering power of blood by 20 dB [12,16]. The following techniques of visualization belong to this particular category.

- **Conventional B-mode**: B-mode imaging with conventional equipment is improved with the addition of MBs, especially in the field of cardiology. The strong backscattering power that MBs introduce, provides images of higher precision and contrast, than previously achieved. Visualization of blood vessels within tissue (myocardium), however, remains a challenge [12,16].

- **Conventional Spectral Doppler**: MBs enable the imaging of blood flow of deep vessels, providing an increase of about 20 dB in the echo [12,16].

- **Conventional Color Doppler**: Referring mostly to Color Flow Imaging (CFI) and Power Doppler Imaging (PDI), contrast agents provide an easier demonstration of the larger arteries. In CFI, a color map superimposes the tissue of interest, of which the color of every pixel is proportional to the Doppler shift in that area. In PDI, the color of each pixel is determined by the mean power of Doppler signal in that region. However, several artifacts arise with the use of MBs, such as ‘blooming’ and ‘flash’ artifact [12,16].

- **Contrast Harmonic Imaging (CHI)**: By using a high-pass filter enabling only the second harmonic component to remain, it is possible to get rid of the signal obtained by the tissue, but not from the tissue harmonic component. Amplitude of the transmitting pulses must be large enough to make sure that useful signals with a second harmonic component will be generated by the MBs, without however destroying the MBs. Transmitting at low frequency is necessary to ensure that both the fundamental frequency and the second harmonic are within the bandwidth of the transducer. The
short echo bandwidth as well as the overlapping of the fundamental and second
harmonic parts of the signals establish limitations to the axial resolution and sensitivity
of the method, although contrast is indeed enhanced [12,16].

- **Harmonic Doppler Imaging:** A combination of the previous technique (CHI) and CFI or
  PDI. A high pass filter is used on the CFI/PDI method, in order to get rid of the
  fundamental component of the signal. ‘Flash’ and ‘blooming’ artifacts are reduced, but
temporal and spatial resolution remain at low levels [12,16].

- **Intermittent Imaging techniques:** These techniques are developed to visualize the
effect of collapsing the MBs on the image. B-mode, Color Doppler or Power Doppler
can be used in combination with disrupting the MBs, providing great sensitivity of
imaging MBs at the cost of low axial resolution and lack of real-time imaging [12,16].

### A.5.2. Non-Destructive Imaging Techniques

Non-Destructive imaging techniques are based on the principle that by using two (or more)
transmission pulses, it is possible to remove the fundamental component (or others) of the
echo signal, keeping only the component of interest (e.g. second harmonic). MBs are not
destroyed, giving the opportunity of real-time imaging, while sufficient energy is introduced
to generate harmonics from the MBs, higher than those generated from the tissue,
resulting in higher contrast-to-tissue ratio. Limitations of these techniques are: the lower
frame rate and the need for minimal movement in comparison with the conventional
imaging methods due to the need of at least two transmitting pulses, as well as the lower
sensitivity in comparison with the high-MI techniques. The following techniques of
visualization belong to this particular category.

- **Pulse Inversion (PI) with two transmissions:** Two short pulses are transmitted one after
  the other, with the second having inversed polarity of the first one. The echoes from
  the two pulses are then added together. This results in eliminating the linear
components of the echo signals (e.g. those generated from the tissue), keeping only
non-linear components of the echo signal, which are mainly generated by the MBs
(Figure 2). In addition, when a transmission pulse is inverted, the second and even
harmonics are the same, while the fundamental and odd harmonics are inverted. Thus, by using this method, one can get rid of the echoes from linear targets, as well as the fundamental and odd components of the echo signal, while at the same time the magnitude of the second and even harmonics is doubled. The main advantage of this technique in comparison with CHI, is the fact that it eliminated the problem of overlapping between the fundamental and second harmonic components of the echo signal, allowing large bandwidth pulses to be used, improving the axial resolution. Limitations include the lower frame rate and the need for minimum movement of the tissue [12,16].

- **Pulse Inversion on Alternate Scan Lines:** The limitation of frame rate that the previous technique introduces (need for 2 pulses to be transmitted along a single line), can be overcome by using the PI technique on alternate scan lines. The transmitted pulse is inverted in every pair of adjacent lines, resulting in the cancellation of echoes from the tissue (linear echoes). However, in this case the fundamental frequency is not perfectly cancelled, since not all points generated from a linear target are the same between a pair of echoes, since the produced sum line can be considered to be an artificial middle one [12,16].

- **Pulse Inversion with three Transmissions:** The problems arising with tissue movement when using PI with two transmissions can be exceeded by using three transmission pulses, with the first and the third being the same while the second has an inverted polarity and twice the amplitude. Summing the echoes from all three has the same results regarding the echo signal as the PI with two transmissions, while the average of the 1st and 3rd echo sequences takes into account any tissue motion [12,16].

- **Harmonic Detection by Coded Excitation:** The principle of this technique are long pulses, which contain changes of either frequency or phase or both. Long pulses enable a greater sensitivity to be achieved, combined together with the MBs. These changes of frequency or phase operate as a code and are detected by a 'matched' filter, which then ‘squeeze’ the long pulses into shorter ones, with higher amplitude. Binary coding and chirp coding are the most common techniques of harmonic detection by coded excitation. In the first method, one pulse consists of several base pulses, which are connected altogether one after the other. A digital code is given binary mode, depending on the method used. For instance, creating such a pulse of
three base pulses, the third one with inverted polarity, created the binary code 1,1,-1 (supposing 1 is for non-inverted pulse and -1 is for inverted). This code is used as reference for decoding the echoes. A perfect match gives a high output (short strong signals), while a possible mismatch gives a lower output. In Chirp coding, the frequency and amplitude of the long pulse varies over its cycles. The filter uses a reference version of the pulse to decode the echoes produced. The closer the shape of the echoes to the transmitted pulse, the higher the output. That results, as previously, in high output for every match, but low output in the case of mismatch or non-coded noise [12,16].

- **Amplitude Modulation:** The simple case is when two pulses with identical waveforms are transmitted, with one have double the amplitude of the other. The echo from the lower amplitude pulse is subtracted by the higher one, but only after it has been amplified first by a factor of two. In this way, less harmonics are generated by the lower amplitude pulse comparing with the high amplitude one, thus after subtraction echoes from stationary tissue are cancelled, leaving only the echoes by the MBs. In comparison with the PI technique, the use of lower frequency of the echo reduces the attenuation resulting in higher sensitivity, while second harmonics generated from the stationary tissue are rejected efficiently [12,16].

- **Contrast Pulse Sequence (CPS) Imaging:** This technique refers in general to any combination of the PI and the Amplitude Modulation. Two or more pulses can be used for transmission, selecting their amplitude and phase accordingly, so that only the harmonic of interest is preserved, while others are rejected [12,16].
A.5.3. Destructive Imaging Techniques

Higher sensitivity can be achieved by using destructive imaging techniques, which make use of high amplitude transmission pulses to burst the MBs. The differences in echo signals before and after destruction are then recorded and useful information can be retracted from them, at the expense of low frame rate and (in some cases) lack of real-time imaging. One very common application of the Non-destructive imaging combined with MBs is the estimation of myocardial perfusion [12].
A.5.4. Ultraharmonic and Subharmonic Imaging

In addition to the generation of the second and higher harmonics (2f, 3f…), when an ultrasound insonates MBs at a frequency f, the nonlinear oscillation of them will also produce components at the ultraharmonic (3f/2, 5f/2…) and subharmonic (f/2, f/3…) regions (Figure 3) [22,24]. In fact, research has shown that generation of components at these regions is almost entirely due to the UCA, which could prove to be a great advantage in ultrasound imaging [23,24].

- **Ultraharmonic Imaging**: The most common application of this technique is the 1.5 harmonic imaging. This method is based on the idea that the MBs signal is much larger in that region (1.5 harmonic), compared to the signal from the tissue. By using the 1.5 harmonic imaging, one can further suppress the response from the linear tissue and even improve the sensitivity, as the 1.5 harmonic frequency is closer to the center of the transducer response, where efficiency of the transducer is maximum. Filtering is usually used to eliminate the responses at the fundamental and second harmonic regions, leaving only the one at 1.5 harmonic. The results are images of higher CTR than fundamental or second harmonic imaging. Combination of this technique with others mentioned here, is currently under investigation [16,21-23].

- **Subharmonic Imaging**: Insonation of MBs at a central frequency has shown to generate echoes at lower frequencies than the fundamental, mainly at half (subharmonic frequency). The reasons behind this phenomenon are believed to be the nonlinear response of the bubbles, as well as the continuous oscillation of them at the central frequency, known as ringing [16]. The amplitude of the echoes in the frequency spectrum is in some cases even greater than the one at the second harmonic. This fact, in combination that the tissue does not generate components at this region, could prove to be a great advantage for CEUS imaging. Similar to the ultraharmonic imaging technique, filtering is usually used for eliminating the components at other regions than the subharmonic, while the echoes are received at half the fundamental frequency. High CTR can also be achieved by using this method, while it is currently under investigation how this technique could be combined with others [16,21-25].
Figure 9: Spectra of scattered signals from SonoVue microbubbles, insonified at frequency 4 MHz [22].
References


