A new ultrasound-based approach to visualize target specific polymeric contrast agent

Malin Larsson¹, Anna Bjällmark¹, Matilda Larsson¹, Kenneth Caidahl², Reidar Winter¹,² and Lars-Åke Brodin¹

1) Department of Medical Engineering, School of Technology of Health, Royal Institute of Technology, Stockholm, Sweden.  
2) Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden.  
3) Department of Cardiology, Karolinska Institutet, Stockholm, Sweden.  
malinl@sth.kth.se

Abstract—There are advantages of using a polymeric shelled contrast agent (CA) during ultrasound imaging instead of lipid shelled CA, e.g. particles can be attached to the surface, which enables an introduction of antibodies to the surface making the CA target specific. For this application it is essential to have a sensitive imaging technique suitable for polymeric CA. However, previously presented results have indicated difficulties in visualizing polymeric CA with commercially available contrast algorithms. Therefore a new subtraction algorithm (SA), was developed that define the difference between contrast and reference images. The aim of this study was to evaluate the response from a polymeric CA, when using the SA and compare it with existing contrast algorithms. Moreover, the possibility to detect a thin layer of CA was tested using the SA.

Ultrasound short-axis images of a tissue-mimicking vessel phantom with a pulsating flow were obtained using a GE Vivid7 system (M12L) and a Philips IE33 system (S5-1). Repeated (n=91) contrast to tissue ratios (CTR) calculated at various mechanical index (MI) using the contrast algorithms pulse inversion (PI), power modulation (PM) and SA at a concentration of 10¹⁸ microbubbles/ml.

The developed SA showed improvements in CTR compared to existing contrast algorithms. The CTRs were -0.99 dB ± 0.67 (MI 0.2), 9.46 dB ± 0.77 (MI 0.4) and 2.98 dB ± 0.60 (MI 0.8) with PI, 8.17 dB ± 1.15 (MI 0.2), 15.60 dB ± 1.29 (MI0.4) and 11.60 dB ± 0.73 (MI 0.8) with PM and 14.97 dB ± 3.97 (MI 0.2), 20.89 dB ± 3.54 (MI 0.4) and 21.93 dB ± 4.37 (MI 0.8) with the SA. In addition to this, the layer detection, when using the SA was successful.

Index Terms— Contrast to tissue ratio, Target specific Ultrasound contrast agent, Vessel mimicking phantom.

I. INTRODUCTION

Ultrasound contrast imaging is frequently used in clinical applications to enhance the visualization of blood flow. A contrast agent (CA) is a gas filled microbubble (MB), stabilized by a shell, that can be intravenously injected into the blood stream via a bolus or a continuous injection. Due to its high scattering ability it can improve the detection of blood when using ultrasound. Hence the perfusion in different organs, such as the myocardium, liver, kidney and tumors, can be quantified [1-2].

The size of the bubble is limited by the diameter of the pulmonary capillaries, which is around 7 µm. In addition to the size limitation, an ideal CA must also be biocompatible, intravenously injectable, stable during the ultrasound examination and have the appropriate acoustic properties in order to increase the backscattering efficiency from the blood compared to the surrounding tissue.

The commercial CAs used in clinical applications today are mainly stabilized by a lipid shell. However, there are advantages of using a polymeric shelled CA instead. Polymeric CA have a longer shelf life, a more narrow size distribution and high ability to carry substances on the shell surface, which enables a multimodal CA and introduction of antibodies to the surface making the CA target specific [3-4]. In the latter case, it is essential to have a sensitive imaging technique optimized for the polymeric CA since the amount of CA to be detected is small. Visualization of inflammation is an example of application for target specific imaging. A future application might also be local release of drugs.

However, previously presented results indicated difficulties in visualizing polymeric CA with commercially available contrast algorithms [5]. The aim of this study was therefore to test, in an experimental setup, whether our recently developed offline subtraction algorithm (SA) can improve the visualization of a polymeric CA compared to existing contrast algorithms. Additionally, the potential of the SA to detect a thin layer of polymeric CA was investigated in an in-vitro setup.

II. METHODS

A. Subtraction algorithm

A subtraction algorithm (SA) was developed in Matlab (MathWorks, Inc. Massachusetts, USA) based on a set of subtractions between gray-scale images: contrast images acquired after injection of CA and reference images acquired before injection of CA. One heart beat was selected for each of the imaging loops using the ECG-curve. A region of interest (ROI) was manually selected in the reference image in the initial frame (ROI size 20 mm x 20 mm). Subsequently, the ROI in the reference image was spatially matched in the corresponding contrast image for each frame using block-matching with normalized cross-correlation as similarity measure. When the best match was found in the contrast image, the areas were subtracted. Pixels having a lower intensity value than zero in the subtracted image were given intensity values of zero. Finally the subtracted image was median filtered in order to remove noise with a filter size of approximately 0.1 mm x 0.4 mm.

To visualize the results from the SA, the subtracted images were converted to a binary image and then color-coded and superimposed in the reference images. The areas in the images containing CA were given a red color, see examples in Fig. 4.
B. Visualization of polymeric shelled ultrasound contrast agents when applying the subtraction algorithm

An experimental setup with a flow phantom mimicking the human carotid artery was built in order to evaluate the SA’s ability to improve the visualization of polymeric CA in comparison to existing contrast algorithms.

Flow phantom construction

A tissue mimicking flow phantom including an embedded vessel in surrounding tissue was designed, see Fig. 1. To construct the vessel, a water solution of polyvinyl alcohol (PVA) (15 %, by mass) (Sigma-Aldrich, St. Louis, MO) and graphite powder (3 %, by mass) with particle size < 50 μm (Merck KGaA, Darmstadt, Germany) was heated to 90°C. The graphite powder was added as acoustic scatterers [6].

After filling a cylindrical vessel mould made of acrylic plastic (inner diameter 6 mm, outer diameter 12 mm, length 100 mm) with the PVA/water solution, it was stored in a freezer at -20°C for 12 h and during the subsequent 12 h it was kept in room temperature (20°C). In total, the phantom underwent three freeze and thaw cycles in order to achieve the appropriate acoustic properties [7]. The vessel phantom was attached in a PVC box (100 mm x 60 mm) covered with a 3 mm thick rubber layer at the bottom, using plastic connectors. As surrounding tissue, a water solution of agar (3 %, by mass) (Merck KGaA, Darmstadt, Germany) and graphite powder (4 %, by mass) with particle size < 50 μm (Merck KGaA, Darmstadt, Germany) was heated to 85°C and then poured into the PVC box. To avoid dissolving of the vessel phantom, the solution was cooled before pouring it into the PVC box. Moreover, to prevent the tube from collapsing, the vessel was filled with de-ionized water before agar solution was poured into the PVC box.

Experimental setup

The phantom was connected to a programmable pump, CompuFlow 1000MR, (Shelley Medical Imaging Technologies, Ontario, Canada) which generated a carotid flow profile with a peak flow of 20 ml/s. A solution containing 40 % glycerol (99 % pure, molecular weight 92.09 g/mol; Sigma-Aldrich, St. Louis, MO) and 60 % de-ionized water was used as blood mimicking solution. A polymer shelled CA, previously described in literature [8], was included in this study. By diluting the master batch of the CA with the blood mimicking solution, a concentration of 10⁵ MB/ml was achieved.

Before the experiment started a drain procedure was performed to remove air bubbles in the blood mimicking solution. Continuously stirring of the blood mimicking solution was achieved by an onboard magnetic stirrer.

Data acquisition and analysis

Ultrasound short-axis loops of the tissue mimicking flow phantom were obtained for different ultrasound systems and imaging techniques, see Table 1. The focus point was fixed to the middle of the lumen for all techniques. In order to obtain an optimal view of the vessel, imaging settings as time gain compensation (TGC), dynamic range, and speckle reduction were adjusted for each imaging technique before data acquisition started. Data for the SA were obtained using a Vivid7 ultrasound system (GE Healthcare, Horten, Norway) in conventional gray-scale mode. Reference and contrast images were stored. The pump triggered an ECG signal at a specific time in the simulated carotid flow profile to enable aligning between the reference and contrast images.

Repeated (n=91 frames) CTRs were calculated at mechanical index (MI) ranging from 0.2 to 0.8 using the contrast algorithms pulse inversion (PI), power modulation (PM) and the new developed SA. The ROIs for the CTR calculations were manually placed, in the center of the vessel lumen and in the surrounding tissue, both at the same image depth. The area of the ROIs was approximately 7 mm². In order to minimize variation when placing the ROI, the same operator performed this work for all three techniques. The ratio between the mean intensity within the ROIs from the vessel lumen and tissue was calculated and presented as CTR.

C. Detection of a thin layer of polymeric shelled ultrasound contrast agents using the subtraction algorithm

An experimental setup was built with a similar vessel flow phantom as previously described to preliminary investigate the potential of the SA to detect a thin layer of target specific polymeric CA.

Phantom construction

The same phantom mould as used in the CTR measurements was included in this experiment. However, preparations of the phantoms differed somewhat, since this phantom had no surrounding tissue.

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tbody>
<tr>
<td>IMAGING TECHNIQUES AND SETTINGS USED FOR THE CONTRAST TO TISSUE RATIO CALCULATIONS</td>
</tr>
<tr>
<td>Ultrasound system</td>
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<tr>
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<tr>
<td>Vivid7 (M1L), GE</td>
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<tr>
<td>ie33 (85-1), Philips</td>
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<tr>
<td>Vivid7 (M1L), GE</td>
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</table>
Experimental setup

The phantom was submerged in a water bath of de-ionized water, see Fig. 2. Before CA was added, the vessel was filled with de-ionized water making the reference imaging loop possible. Thereafter the vessel was filled with a water solution including CA. Both the previously used polymeric CA and a modified version of it, with superparamagnetic iron oxide nanoparticles embedded in the shell, were included in this experiment [8]. Desired concentrations were achieved by diluting the master batch of the CA with de-ionized water to a concentration of $10^5$ MB/ml for both MB types.

Data acquisition and analysis

Ultrasound long-axis loops of the phantom were obtained using a Vivid7 ultrasound system (GE Healthcare, Horten, Norway) in conventional gray-scale mode with a M12L transducer ($f = 14$ MHz, $MI = 0.4$). The transducer was placed in the water bath underneath the phantom, see Fig. 2. In order to obtain an optimal view of the vessel, imaging settings as TGC, dynamic range, and speckle reduction were adjusted. The focus point was fixed at a depth in the middle of the lumen. A reference image was acquired before the phantom was filled with the water/CA solution. Thereafter, water/CA solution (MB without embedded nanoparticles) was added and the vessel phantom was imaged upside down 6 h after the introduction of the water/CA solution. The CA was then rising towards the upper part of the vessel (towards posterior wall in the ultrasound image) since no flow was used in the setup. A drain procedure was then applied to clean the hoses to the phantom from MB, which was followed by filling the phantom with a new water/CA solution (MB with embedded nanoparticles). Thereafter, a magnet was placed on the top of the phantom, to reinforce the rising effect due to the magnetic properties of the modified CA. Long-axis images of the phantom were obtained, in a upside down position, 10 min after placing the magnet on the top of the phantom.

The use of a static phantom instead of a flow phantom implied no pulsating movements of the phantom, making the use of a triggered ECG signal unnecessary. Only the speckle alignment between the reference and contrast images was used for the subtraction of the two loops (reference and contrast) when analyzing data with the SA. The subtracted images were converted to binary images, based on a threshold of one. The binary images were color-coded and superimposed in the reference images.

### III. RESULTS

The results from the CTR measurements for all imaging techniques are listed in Table 2 whereas Fig. 3 shows examples of short-axis ultrasound images of the phantom for the different imaging techniques.

Fig. 4 shows examples of long-axis images of the phantom with a thin layer of CA close to the posterior wall. The detected CA was color-coded in red in the images.

### IV. DISCUSSION

The results of this study show high potential for our recently developed SA in the visualization of polymeric CA. Previous studies have revealed difficulties in the visualization of polymeric CA when using commercially available contrast algorithms, which the results from this study confirm [5]. When the SA was applied, the detection of polymeric CA was improved compared to existing contrast algorithms.

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**Fig. 2.** Schematic illustration of the experimental setup for the layer detection experiments with the vessel phantom imaged in a water-filled box.

**Fig. 3.** Zoomed ultrasound short-axis images of a vessel phantom containing blood mimic and polymeric CA. a) conventional gray-scale image (MI=0.4, $f=14$ MHz), b) Pulse inversion image (MI=0.4, $f=5/10$ MHz), c) Power modulation (MI=0.4, $f=1.5/3.2$ MHz), d) Subtraction algorithm image (MI=0.4, $f=14$ MHz).

**Table 2**

<table>
<thead>
<tr>
<th>Imaging Technique</th>
<th>MI 0.2</th>
<th>MI 0.4</th>
<th>MI 0.8</th>
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<tbody>
<tr>
<td>Pulse inversion</td>
<td>-0.99</td>
<td>9.46</td>
<td>2.98</td>
</tr>
<tr>
<td></td>
<td>(0.67)</td>
<td>(0.77)</td>
<td>(0.60)</td>
</tr>
<tr>
<td>Power modulation</td>
<td>8.17</td>
<td>15.60</td>
<td>11.60</td>
</tr>
<tr>
<td></td>
<td>(1.15)</td>
<td>(1.29)</td>
<td>(0.73)</td>
</tr>
<tr>
<td>Subtraction algorithm</td>
<td>14.97</td>
<td>20.89</td>
<td>21.93</td>
</tr>
<tr>
<td></td>
<td>(3.97)</td>
<td>(3.54)</td>
<td>(4.37)</td>
</tr>
</tbody>
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*Mechanical Index (MI), Mean (n=91) CTR (dB) with corresponding standard deviation (dB) in brackets.*
The poor performance of the existing contrast algorithms in visualizing the polymeric CA might be due to the large shell thickness of the polymeric CA in comparison with commercially available CA [9]. The thick shell will increase the shear viscosity and the shear modulus of the encapsulated bubble, which in turn results in increased damping of the bubble oscillation and hence a less well-defined resonance frequency. Available contrast algorithms are developed for commercial CAs with a thin, flexible shell, allowing for large radius excursions. Such shell gives rise to a well-defined resonance frequency which is utilized to obtain nonlinear echoes for conventional contrast pulse sequences.

According to the performed experiments the detection of a layer of CA was possible when using the SA, which is crucial for a target specific CA. These results indicate that the SA might be a helpful tool in the detection of adhesion target specific CA to a particular area, such as atherosclerotic plaques. However, only a relatively high concentration of CA was applied in this experiment. Hence, the thickness of the layers might have been too large to mimic the adhesion of target specific CA to in-vivo tissue. On the other hand, the two developed experimental setups, i.e. rising effect with and without influence of a magnet, have shown to be suitable for layer detection experiments and could possibly be refined in future studies. Therefore, more extensive in-vitro and in-vivo experiments are needed to further investigate the potential of the SA to detect thin layers of target specific CA.

Although the results from this study are promising, one must remember that there are also limitations with the described algorithm. For example, the SA requires a reference image, obtained at a similar position as the contrast image. Therefore, movements of the analyzed object can cause speckle decorrelation between the two subtracted images, resulting in difficulties to align the images. However, with the use of an ECG triggering signal, the impact of regular movements occurring during the cardiac cycle can be minimized. On the other hand, movements of the patients itself or the transducer during image acquisition cannot be adjusted for. As the SA is an offline method, data need to be processed before the subtracted image can be obtained. In some cases this might result in insufficient image quality due to the lack of direct feedback during image acquisition. However, as both the reference and contrast images are stored under optimal conditions, this error is most likely negligible in the vast majority of cases.

The controlled setup, in which the SA was tested, made it possible to visually assess whether the SA was able to detect true areas in the image containing signals from the CA. In clinical applications, the setting is more challenging. The regions perfused with blood are small and more difficult to image. However, the promising results from this study can be seen as a preliminary validation of the algorithm.

V. CONCLUSION

The developed SA showed high potential to visualize polymeric CA, as expressed by higher CTR values compared with existing contrast algorithms. It was also preliminary showed that detection of a layer of CA was possible, which is essential for future applications in target specific imaging. However, both CTR measurements and layer detection experiments are needed in larger studies in order to establish the feasibility and accuracy of the new technique. Moreover, the SA needs to be evaluated in-vivo.

REFERENCES