ULTRASOUND ATTENUATION ESTIMATION IN HARMONIC IMAGING FOR ROBUST FATTY LIVER DETECTION

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Abstract—Accurate detection of liver steatosis is important for liver disease management. Ultrasonic attenuation coefficient estimation (ACE) has great potential in quantifying liver fat content. The commonly used ACE methods (e.g., spectral shift methods, reference phantom methods) assume linear tissue response to ultrasound and were developed in fundamental imaging. However, fundamental imaging may be vulnerable to reverberation clutters introduced by the body wall. The clutters superimposed on liver echoes may bias the attenuation estimation. Here we propose a new ACE technique, the reference frequency method (RFM), in harmonic imaging to mitigate the reverberation bias. The accuracy of harmonic RFM was validated through a phantom study. In a pilot patient study, harmonic RFM performed more robustly in vivo compared with fundamental RFM, illustrating the potential of ACE in harmonic imaging. (E-mail: Chen.Shigao@mayo.edu) © 2020 World Federation for Ultrasound in Medicine & Biology. All rights reserved.

Key Words: Fatty liver detection, Ultrasound attenuation estimation, Harmonic imaging.

INTRODUCTION

Fatty liver, or hepatosteatosis, is characterized histologically by triglyceride accumulation within the cytoplasm of hepatocytes and refers to fat accumulation in the liver exceeding 5%—10% by weight (Obika and Noguchi 2012). When hepatosteatosis is present in the absence of excessive alcohol consumption, it is termed non-alcoholic fatty liver disease (NAFLD). NAFLD is the most common liver disorder in Western countries, affecting 17%—46% of adults (Marchesini et al. 2016). Between 75 million and 100 million individuals in the United States are estimated to have NAFLD (Rinella 2015). About 20%—30% of patients with NAFLD will develop a more severe form called non-alcoholic steatohepatitis (NASH), which may result in liver fibrosis and progress to cirrhosis, liver failure or hepatocellular carcinoma (Machado and Cortez-Pinto 2013). Therefore, detection of NAFLD is important for diagnosis of NASH at an early stage for timely intervention to improve long-term outcome. Liver biopsy is the gold standard for diagnosis and staging of NAFLD (Machado and Cortez-Pinto 2013). However, the invasiveness of liver biopsy makes it unsuitable for screening and frequent follow-ups. Non-invasive alternatives such as B-mode ultrasound and computed tomography (CT) have low sensitivity for liver steatosis (Dasarathy et al. 2009; Lawrence et al. 2012). Proton density fat fraction (PDFF) acquired with magnetic resonance imaging (MRI) is a standardized and objective measure of mobile proton density proportion attributable to fat in the liver and has been reported to have high fat measurement accuracy in phantoms, human liver samples and animal and human studies (Kinner et al. 2016). The imaging time and cost have also been considerably reduced with abbreviated sequences (Canellas et al. 2019). However, MRI has limited availability worldwide with more restrictions and contraindications, thus limiting its use in clinical practice. Therefore, low-cost, widely accessible and accurate liver steatosis staging biomarkers are urgently needed. It has been reported that the elevated fat content in the liver is associated with increased ultrasound attenuation, and several studies have reported the feasibility of ultrasound attenuation in steatosis staging using Fibroscan’s controlled attenuation parameter
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(CAP) (Taylor et al. 1986; Lin et al. 1988; Lu et al. 1999; Sasso et al. 2010; Karlas et al. 2017). However, CAP is not compatible with clinical ultrasound scanners and requires an expensive standalone device, which could limit its accessibility. In addition, measurements are made blindly without B-mode imaging guidance and, thus, are susceptible to bias caused by major vessels within the liver. Consequently, attenuation coefficient estimation (ACE) technologies that are compatible with clinical ultrasound scanners would have high clinical significance.

At present, the two commonly used ACE methods in ultrasound array imaging systems are the spectral shift method (Kuc and Li 1985; Bigelow et al. 2008; Samimi and Varghese 2015) and the reference phantom-based methods (Kuc and Schwartz 1979; Kuc 1980; Parker and Waag 1983; Parker et al. 1988; Yao et al. 1990; Kim and Varghese 2008; Coila and Lavarrello 2018). The spectral shift method estimates the attenuation coefficient through the downshift of the ultrasound center frequency with increasing depth (Kim and Varghese 2008). For the reference phantom-based methods (Kuc and Schwartz 1979; Kuc 1980; Parker and Waag 1983; Parker et al. 1988; Yao et al. 1990; Kim and Varghese 2008; Samimi and Varghese 2016), a well-calibrated phantom is needed to normalize system-dependent effects such as focusing, diffraction and time gain compensation (TGC). To our knowledge, the aforementioned ACE methods were developed and implemented under fundamental imaging mode so that they were vulnerable to the reverberation clutters introduced by the body wall. The clutter signals superimposed on liver echoes may bias attenuation estimation in liver, especially for patients with a large body mass index (BMI). In contrast, harmonic imaging can effectively mitigate the reverberation issue compared with fundamental mode, which makes it the default mode for abdominal scanning in many commercial ultrasound scanners (Krishnan et al. 2017). The reduction of reverberation clutters in harmonic imaging may lead to more accurate ACE measurements of liver.

Recently, we developed a novel system-independent ACE technique based on spectra normalization, which is referred to as reference frequency method (RFM). This technique does not require a reference phantom for normalization. The power of each frequency component is normalized by the power of an adjacent frequency component in the spectrum to cancel system-dependent effects such as focusing and TGC (Gong et al. 2019). In this article, we propose use of the RFM in harmonic imaging (RFM-HI) for more robust ACE in liver. The accuracy of RFM-HI was first validated with a calibrated tissue-mimicking phantom. The feasibility of in vivo RFM-HI was tested on 20 patients who underwent clinically indicated MRI of liver. The acquired attenuation estimates were correlated with MRI-PDFF to evaluate the performance of RFM-HI.

METHODS

Theory

In ultrasound harmonic imaging, the power spectrum of the backscattered signals $S(f_i, z)$ can be modeled as a function of backscatter location and the frequency of ultrasound (Yao et al. 1990; Nam et al. 2011)

$$S(f_i, z) = G(f_i) \cdot TGC(z) \cdot D(f_i, z) \cdot H(f_i, z) \cdot BSC(f_i) \cdot A(f_i, z)$$

(1)

where $G(f_i)$ accounts for the transmit and receive transducer responses at frequency $f_i$ ($i$ is the frequency component within the second harmonic bandwidth); TGC($z$) is the TGC, which varies as a function of depth $z$; $D(f_i, z)$ is the combined effects of focusing, beamforming and diffraction; $H(f_i, z)$ accounts for the harmonic generation during ultrasound propagation; BSC($f_i$) is the backscatter coefficient, which is assumed to be uniform in the local region of interest (ROI); and $A(f_i, z)$ is the frequency-dependent attenuation defined as (Yao et al. 1990; Nam et al. 2011)

$$A(f_i, z_k) = \exp(-4af_i z_k)$$

(2)

where $a$ is the frequency-dependent ultrasound attenuation coefficient. $A(f_i, z)$ is also assumed to be uniform in the ROI and has linear frequency dependency.

In the RFM, we assume that the differences in beamforming and diffraction effects between two adjacent frequency components ($i.e., f_i$ and $f_{i+1}$) are negligible as $D(f_i, z) = D(f_{i+1}, z)$. Then the power spectra can be normalized by calculating the power ratio ($Rs(f_i, z)$) between adjacent frequency components $S(f_i, z_k)$ and $S(f_{i+1}, z_k)$ to cancel TGC and diffraction as

$$Rs(f_i, z) = \frac{S(f_i, z)}{S(f_{i+1}, z)} = \frac{G(f_i)}{G(f_{i+1})} \cdot \frac{TGC(z)}{TGC(z)} \cdot \frac{D(f_i, z)}{D(f_{i+1}, z)} \cdot \frac{H(f_i, z)}{H(f_{i+1}, z)} \cdot \frac{BSC(f_i)}{BSC(f_{i+1})} \cdot \frac{A(f_i, z)}{A(f_{i+1}, z)}$$

(3)

We assume that the differences in harmonic generation effects between two adjacent frequency components are also negligible as $H(f_i, z) = H(f_{i+1}, z)$; we then obtain

$$Rs(f_i, z) = \frac{G(f_i)}{G(f_{i+1})} \cdot \frac{BSC(f_i)}{BSC(f_{i+1})} \cdot \frac{A(f_i, z)}{A(f_{i+1}, z)}$$

(4)

After taking the natural logarithm on both sides of eqn (4), we obtain the linear relationship between

$$\ln(Rs(f_i, z)) = \ln(G(f_i)) - \ln(G(f_{i+1})) + \ln(BSC(f_i)) - \ln(BSC(f_{i+1})) - \ln(A(f_i, z)) + \ln(A(f_{i+1}, z))$$

(5)
frequency power ratio \(\ln[Rs(f_i, z)]\) and imaging depth \(z\):
\[
\ln[Rs(f_i, z)] = -4a(f_i-f_{i-1})z + \ln \left[ \frac{G(f_i)}{G(f_{i-1})} \cdot \frac{BSC(f_i)}{BSC(f_{i-1})} \right]
\]

Then the attenuation coefficient can be estimated from the slope of the decay trend of frequency power ratio with respect to each second harmonic frequency component:
\[
a_i = \frac{\text{slope}}{-4(f_i-f_{i-1})z}
\]

Multiple frequency power ratios can be averaged within the second harmonic frequency bandwidth to facilitate more robust attenuation estimation.

**Tissue-mimicking phantom validation**

The proposed RFM-HI was first validated on a tissue-mimicking phantom calibrated using the 2-D ultrasound attenuation imaging (ATI) function on a commercial ultrasound scanner, the Apio i800 (Canon Medical Systems, Tochigi, Japan). ATI acquisitions were performed using the curved array i8 CX1 (1–8 MHz) according to regular acquisition procedures (Jeon et al. 2019). The median attenuation coefficient measured from the phantom was 0.57 dB/cm/MHz from five repeated valid measurements \((R^2 \geq 0.90, \text{interquartile range/median <30%})\) (Jeon et al. 2019). Harmonic RFM data of the calibrated phantom were acquired with a General Electric LOGIQ E9 (LE9) system (General Electric Healthcare, Wauwatosa, WI, USA) with conventional line-by-line focused beam scanning and the curved array transducer C1-6 D (1–6 MHz, General Electric Healthcare). For LE9 harmonic imaging, 100 A-lines were acquired with focal depth set at 6.5 cm. The imaging frame rate was 20 Hz. The center frequency of the transmit pulse was around 2 MHz. IQ data obtained from the calibrated phantom were stored using the RF Capture module available on the LE9 and then offline-processed for attenuation estimation. The IQ post-processing method was similar to that described in Gong et al. (2019). Briefly, a ROI was selected on the beamformed IQ image that was set axially around 2–10 cm with 40 lateral A-lines for the phantom study. The selected ROI was divided into 20-wavelength-long data blocks along the axial direction with 90% overlaps (the wavelength was calculated with respect to the second harmonic center frequency, \(i.e., 4 \) MHz). Each A-line segment in a given data block was first zero-padded and then Fourier transformed to obtain a single power spectrum. The power spectra of all A-line segments in the given data block were averaged laterally to obtain the mean power spectrum at a certain depth. The frequency power ratio between adjacent frequencies was calculated using eqn (3). Multiple frequency power ratios \(\ln[Rs(f_i, z)]\) with respect to each second harmonic frequency component \(f_i\) were averaged within the frequency bandwidth of 3.2–4.5 MHz to provide a mean frequency power ratio. At last, linear regression was applied on the linearly decaying portion of the mean frequency power ratio curve for attenuation estimation.

**In vivo liver test**

For clinical validation, the proposed method was tested on 20 patients, who underwent clinically indicated MRI of the liver (9 males, 11 females; age: 56 ± 10 y; BMI: 32.4 ± 6.3 kg/m²). The study was approved by the institutional review board of Mayo Clinic. Written informed consent was obtained from each participant at the time of enrollment. All patients were fasted more than 6 h before scanning. PDFF acquired with MRI was used as reference standard (Kinner et al. 2016). The PDFF was measured with the MRI scanner GE Optima 450 (General Electric Healthcare) using the IDEAL IQ sequence. The in vivo liver ultrasound scans were performed on the same day as MRI-PDFF, using the same GE-LE9 system and C1-6 D probe used in the phantom study. All other imaging parameters were the same as described above. Other than harmonic imaging, RFM in fundamental imaging (RFM-FI) was also performed for the 20 patients for comparison. For the LE9 fundamental mode, 127 A-lines were acquired with the same focal depth at 6.5 cm as in HI. The imaging frame rate was 28 Hz with a pulse center frequency around 3 MHz. The frequency range used for RFM-FI was 2.5–3.5 MHz.

**Construction of 2-D attenuation coefficient maps for in vivo livers**

For in vivo ACE under both fundamental and harmonic imaging modes, an approximately \(5 \times 5\)-cm ROI was first selected for attenuation evaluation at around 4–10 cm of depth. Laterally, the ROI was positioned in the most uniform liver parenchymal area from the liver right lobe B-mode images. Rib shadow, major vascular structures and cysts were avoided during ROI selection (Taylor et al. 1986). The selected ROI was then divided into multiple \(3 \times 3\)-cm sub-ROIs, with 70% overlap in both the lateral and axial directions. ACE analysis was performed within each sub-ROI according to the aforementioned steps. The estimated attenuation value was assigned to each pixel inside the corresponding sub-ROI. Then the sub-ROI was translated in both the lateral and axial directions to repeat the same ACE process until it covered the entire ROI. The local attenuation coefficient of each pixel was calculated as the
average of all estimated attenuation values obtained from all sub-ROIs covering that pixel. The degree of attenuation was color-coded to form the 2-D ACE map which was overlain on the corresponding B-mode regions. The mean attenuation value of the 2-D ACE map was calculated and displayed in units of dB/cm/MHz for each measurement. The median attenuation value from the 10 consecutive measurements was used as the final attenuation estimation for each patient. At last, the final attenuation measurements from all patients under fundamental and harmonic imaging were correlated with clinically indicated MRI-PDFF to evaluate the performance of the proposed ACE method under both imaging modes.

**RESULTS**

In Fig. 1 are the mean frequency power curve and mean frequency power ratio curve after taking the natural logarithm. Linear regression was applied on the linearly decaying portion of the mean frequency power ratio curve (i.e., 4–10 cm in Fig. 1b), and an attenuation coefficient of 0.59 dB/cm/MHz was estimated from the decay slope. The process was repeated on five measurements, and a mean attenuation coefficient of 0.59 dB/cm/MHz was obtained with a standard deviation of 0.03 dB/cm/MHz. When these five RFM-HI phantom measurements were compared with Toshiba calibrations, no significant difference was detected using a two-tailed $t$-test at a significance level of 5%. The phantom study validated that accurate ACE values can be obtained using RFM under harmonic imaging mode. Note that RFM-HI could not be used to obtain the linear decay trend in the near field of the frequency power ratio curve (i.e., <4 cm of depth); this will be discussed in detail in the Discussion.

In Fig. 2 are representative 2-D ACE maps acquired under both fundamental and harmonic imaging modes from three patients with different MRI-PDFF values. In these cases, RFM-FI and RFM-HI provided similar ACE performances, and both agreed well with MRI-PDFF, illustrating the feasibility of RFM in harmonic imaging *in vivo*. The demographic data of all 20 patients are summarized in Table 1, including mean ROI sizes, body wall thicknesses and median attenuation values from the 10 consecutive measurements under RFM-FI and RFM-HI. The correlation plots of MRI-PDFF versus median ACE estimations are provided in Fig. 3. Harmonic RFM had improved correlation with PDFF values (coefficient of determination, $R = 0.91$, $p < 0.05$) compared fundamental RFM ($R = 0.68$, $p < 0.05$). A significant difference was detected between these two correlation coefficients using Fisher’s Z-transformation ($p < 0.05$). The improvement indicated the robustness and potential of the RFM in harmonic imaging.

**DISCUSSION**

We have described the process of applying a newly developed system-independent ACE technique, the RFM, in harmonic imaging. In this study, we assumed that the differences in harmonic generation between adjacent frequencies can be canceled out during spectra normalization. The assumption was first validated by a phantom study. We noticed that such assumption was valid and accurate attenuation values could be obtained at greater imaging depths (i.e., >4 cm in Fig. 1b) rather than at smaller depths (i.e., <4 cm). This may be because in harmonic imaging, the change in backscattered signal magnitude with depth is a competition between harmonic generation and ultrasound attenuation. In the smaller depth region, the generation of a second
harmonic tended to dominate the magnitude change, leading to violation of the assumption. The second harmonic energy accumulated as ultrasound propagated into the medium, and harmonic echo magnitude slowly built up until it reached the peak. Afterward, echo magnitude started to decay at greater depths, where ultrasound attenuation played a more significant role (i.e., attenuation dominating region) and the assumption became valid. Correspondingly, the frequency power ratio curve was linearly attenuated in a similar depth region, and an accurate attenuation value could be obtained. We admit that this is a limitation in that RFM-HI was unable to provide an accurate ACE in the near field where harmonic generation dominated. However, this may not be a critical issue as the first few centimeters of an abdominal ultrasound image usually consists of body walls (see Fig. 2) which are not suitable for analysis. In addition, normal human livers are generally around 13-15 cm in depth in ultrasound images (Riestra-Candelaria et al. 2016). Consequently, the depth range in harmonic imaging should still be sufficient for ACE. In this study, the attenuation-dominating region was experimentally determined in the phantom to be 4 cm or beyond in depth, which was generally also applicable to patient imaging. Note that, in Fig. 2e and 2f, the ROI was placed in the depth range between 3 and 7 cm. This is because the liver fat content for this patient was very high (PDFF = 43%), resulting in high ACE values (>1 dB/cm/MHz). Consequently, attenuation started to dominate at a shallower depth in this case compared with other patients. To avoid noise contamination for signals in deeper imaging regions, the ROI location was shifted upward by 1 cm to ensure sufficient signal-to-noise ratio (SNR). Nevertheless, the sweet spot for robust ACE in harmonic imaging

![Fig. 2. Representative 2-D attenuation coefficient maps acquired under both fundamental and harmonic imaging modes from three patients with different proton density fat fraction (PDFF).](image-url)
generally is around depths of 4–10 cm, where attenuation dominates and the SNR is sufficiently high. In future studies, quality control factors such as the linear coefficient of determination can be applied to automatically determine the starting point of the linear decay portion on frequency power ratio curves and, thus, maximize the available depth range for ACE.

The clinical feasibility of RFM-HI was illustrated in a pilot clinical study with 20 patients. Harmonic RFM-HI provided more robust attenuation estimations and better correlation with the MRI-PDFF compared with fundamental RFM. One possible reason for the enhanced ACE performance in harmonic imaging may be attributed to the suppression of reverberation clutters originating from the body wall. In Figure 4 are fundamental and harmonic ultrasound images of water below a pork belly sample (the pork belly was warmed to 37˚C before imaging). Ideally, the water should have no echoes. However, the fundamental image

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex</th>
<th>Body mass index</th>
<th>Age</th>
<th>MRI-PDFF</th>
<th>ROI size (cm) (lateral x axial)</th>
<th>Body wall thickness (cm)</th>
<th>Attenuation coefficient estimation</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>Male</td>
<td>29.2</td>
<td>39</td>
<td>9%</td>
<td>5.5 x 5.1</td>
<td>2.8</td>
<td>0.64 ± 0.047 0.64 ± 0.029</td>
</tr>
<tr>
<td>2</td>
<td>Female</td>
<td>34.27</td>
<td>53</td>
<td>15%</td>
<td>5.8 x 5.3</td>
<td>2.8</td>
<td>0.43 ± 0.027 0.67 ± 0.009</td>
</tr>
<tr>
<td>3</td>
<td>Female</td>
<td>25.97</td>
<td>57</td>
<td>43%</td>
<td>6.4 x 4.8</td>
<td>2.2</td>
<td>1.05 ± 0.019 1.01 ± 0.044</td>
</tr>
<tr>
<td>4</td>
<td>Male</td>
<td>39.21</td>
<td>71</td>
<td>14%</td>
<td>6.0 x 5.0</td>
<td>2.9</td>
<td>0.73 ± 0.039 0.80 ± 0.032</td>
</tr>
<tr>
<td>5</td>
<td>Female</td>
<td>38.36</td>
<td>53</td>
<td>3.00%</td>
<td>5.7 x 4.9</td>
<td>3.2</td>
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</tr>
<tr>
<td>6</td>
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<td>31.29</td>
<td>53</td>
<td>4.50%</td>
<td>5.4 x 5.4</td>
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<tr>
<td>7</td>
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<td>29.69</td>
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<td>8.50%</td>
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<td>51</td>
<td>11%</td>
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<tr>
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<td>27.5</td>
<td>66</td>
<td>8.50%</td>
<td>5.8 x 5.0</td>
<td>3.1</td>
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<td>5%</td>
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<td>2.6</td>
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<tr>
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<td>63</td>
<td>10.50%</td>
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<td>0.64 ± 0.035 0.70 ± 0.037</td>
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<tr>
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<td>33.05</td>
<td>47</td>
<td>8%</td>
<td>5.6 x 5.6</td>
<td>2.9</td>
<td>0.58 ± 0.051 0.63 ± 0.046</td>
</tr>
<tr>
<td>19</td>
<td>Male</td>
<td>32.8</td>
<td>50</td>
<td>2.50%</td>
<td>5.5 x 5.6</td>
<td>2.2</td>
<td>0.51 ± 0.050 0.55 ± 0.036</td>
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<tr>
<td>20</td>
<td>Female</td>
<td>27.3</td>
<td>73</td>
<td>31%</td>
<td>5.9 x 5.0</td>
<td>2.5</td>
<td>0.87 ± 0.051 0.90 ± 0.027</td>
</tr>
</tbody>
</table>

MRI-PDFF = magnetic resonance imaging proton density fat fraction; ROI = region of interest.

Fig. 3. Correlation plots of estimated ultrasound attenuation coefficients versus proton density fat fraction (PDFF) measured with magnetic resonance imaging (MRI) acquired under both (a) fundamental and (b) harmonic ultrasound imaging for 20 patients. ACE = attenuation coefficient estimation; MRI = magnetic resonance imaging; PDFF = proton density fat fraction.
revealed severe reverberation clutters from the pork belly in water (Fig. 4a). In practice, such clutters from the body wall may superimpose on liver echoes and bias the estimated attenuation values. In the pilot study, patient body wall thickness exhibited a strong and significant correlation with patient BMI ($r = 0.72$, $p < 0.05$). Therefore, the reverberation issue may be exacerbated for high-BMI patients, usually the group at high risk for hepatosteatosis (the mean BMI for patients in the study was $32.4 \pm 6.3$ kg/m$^2$) (Loomis et al. 2016). As a result, the reliability and accuracy of fundamental ACE may be hindered for these high-BMI patients: The residual of each estimated attenuation value with respect to the linear regression line in Figure 3a was calculated as a metric to evaluate the robustness of RFM-FI. A moderate correlation was detected between the fundamental residues and patient BMI ($r = 0.66$, $p < 0.05$). However, no significant correlation was detected between the residues in RFM-HI and patient BMI ($r = 0.22$, $p = 0.35$), possibly because harmonic imaging can suppress reverberation clutters more effectively than fundamental imaging (see Fig. 4). Consequently, harmonic imaging may play a more important role for high-BMI patients when reverberation clutter is high. More extensive clinical studies with larger patient sample sizes are needed to assess the full capability of RFM-HI for fatty liver screening and monitoring. The reverberation artifacts in fundamental imaging may be reduced by rotating the probe to increase the angle between transducer surface and liver capsule during ultrasound scanning, as suggested by Dahl and Sheth (2014). However, this may increase the scanning difficulty and introduce rib shadowing effects or coupling issues.

As mentioned above, one concern with RFM-HI is ultrasound penetration. With new single-crystal transducers and scanner hardware, penetration of ultrasound imaging has improved significantly. The high BMI values of the pilot patient study also reveal the feasibility of using harmonic RFM in difficult-to-image patients. Moreover, extensive studies have been performed to improve the SNR of ultrasound images, using multifocuses or multiple transmit beams (Tong et al. 2014). The delay-encoded harmonic imaging technique as described in Gong et al. (2017) could also be considered to mitigate the penetration issue and facilitate reliable ACE with harmonic imaging. The robustness of the proposed harmonic RFM technique is expected to be further improved after combination with these SNR-improving techniques. In addition, noise cancellation strategies can also be investigated to further suppress the influence of noise on harmonic RFM analysis (Huang et al. 2019).

The ROI used in the study is relatively large (mean ROI size for all patients enrolled in the study: 5.3 cm [min: 4.6 cm, max: 6.0 cm] × 5.8 cm [min: 5.4 cm, max: 6.4 cm]). The ROI size selected was a trade-off among ACE resolution, measurement accuracy and computational cost. From this study, a general ROI size of 5–6 cm in both lateral and axial dimensions can be suggested; this offers a robust solution to estimation of mean attenuation coefficients of relatively uniform tissues such as liver parenchyma. However, RFM may not be suitable for detecting local attenuation variations for heterogeneous tissues, partly because of the low transmit frequencies of curved arrays, which have larger wavelengths and thus require large sub-ROIs for ACE. For measuring and monitoring ACE in other tissues, such as the breast, a linear array probe with higher transmit frequencies can be used for smaller sub-ROIs to detect local ACE changes.

The acquisition and post-processing time for RFM was a little longer compared with that for the ATI real-time examination. The times it took for RFM IQ data storage using the RF Capture module on the LE9 system and IQ data offline processing on a HP EliteDesk 800 G4 computer using MATLAB R2018 b software (The MathWorks, Natick, MA, USA) were around 2 and 1 s, respectively. However, the ACE process for each sub-ROI is independent,
which makes it amenable to parallel programming. Therefore, further optimization can be performed to speed up the RFM process for easier clinical application.

CONCLUSIONS

We have proposed use of a new reference phantom-free ACE method, the RFM, in harmonic imaging. Harmonic RFM provided accurate and more robust attenuation estimation in phantom and *in vivo* liver studies compared with fundamental RFM. The enhancement indicates the feasibility and potential of harmonic RFM in liver steatosis detection, especially for patients with large BMIs.

**Conflict of interest**—The authors declare that there is no conflict of interest regarding the publication of this article.

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