**Response to the Reviewers’ Comments**

Thanks for the editorial and reviewers’ comments. According to the comments, the manuscript has been revised in the following aspects,

1. Swine muscle with a 30-mm thickness has been placed above the phantom to mimic the beam path in the clinical settings. And the evaluation of the targeting accuracy has been performed on the targets with three different sizes. In order to correct the focal shift caused by the tissue layer in the axial direction, the focal correction along the beam path has been employed based on the empirical formula1. Please see 4.7 and 5.2 in PROTOCOL.
2. The over goal of this study has been clearly stated in the section of INTRODUCTION. The sections of PROTOCOL and DISCUSSION have been rephrased, and spelling and grammar issues have been thoroughly addressed. In addition, the abstract in the manuscript has been rewritten.
3. The supplementary material has been added to provide the detailed description of our clinical USgHIFU phased array system. Besides, the drawing of the square model as well as the phantom holder has also been given.

We have considered the comments carefully and provided the response to the reviewers’ comments as below.

**Editorial comments:**

*Changes to be made by the author(s) regarding the manuscript:*

*1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.*

Response: We have proofread the revised manuscript and addressed the spelling or grammar issues.

*2. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. “This figure has been modified from [citation].”*

Response: The method herein has been modified and the new results have been provided, therefore, the figures are different from our previous paper.

*3. Please label/number the institutional affiliation of each author.*

Response: The institutional affiliation of each author has been labeled in the manuscript.

*4. Please rephrase the Introduction to include a clear statement of the overall goal of this method.*

Response: The introduction has been rephrased to be more concise and the overall goal of this method is clear.

*5. Please remove commercial language and use generic terms instead: SolidWorks, Matlab, etc.*

Response: The commercial language has been replaced with generic terms in the manuscript.

*6. 1.1 and 2.1: Schematics of the square model and phantom holder would be helpful.*

Response: Schematic figures of the square model and the phantom holder have been provided in the supplementary material.

*7. After you have made all the recommended changes to your protocol (listed above), please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Please note that design and calculation steps are not appropriate for filming.*

Response: The essential steps of the protocol have been highlighted in red for the video.

*8. References: Please do not abbreviate journal titles.*

Response: The journal titles has been replaced with their full names.

*9. Table of Equipment and Materials: Please sort the items in alphabetical order according to the Name of Material/ Equipment.*

Response: The items in the table of equipment and materials has been sorted in alphabetical order according to the Name of Material/ Equipment.

**Reviewer #1:**  
*HIFU is emerging as a safe and effective treatment modality in the cancer/solid tumor ablation. The calibration of the HIFU system is important for the consistent performance. The authors developed a method to evaluate the targeting accuracy in the focal plane. No significant scientific impact was found here despite a little practice trick. Targeting accuracy in the axial direction is more important. Defocusing through inhomogenous tissue is very critical issue in clinics using certain algorithm to elements for compensating the phase aberration.*

Response: To mimic the beam path in the clinical settings, the protocol of our method has been modified by placing fresh swine muscle with a 30-mm thickness above the phantom. In order to take the focal shift along the axial direction caused by beam path into account, the empirical formula in our previous study1 has been used, and the layer of the placed swine muscle could induce a focal shift of about 3 mm along the beam path according to equation (1).

|  |  |  |
| --- | --- | --- |
|  |  | (1) |

where the focus shift is dependent on *AO* (tissue thickness), *θi* (determined by transducer *F* number), and *θt* (related with the tissue acoustic speed and transducer *F* number). And the result of the empirical formula is comparable to the numerical calculation.

To compensate the focal shift, the focal correction is performed by setting the depth of the focal plane at 3 mm beneath the upper surface of the bovine muscle tissue instead of phase correction. And the result without focal correction shows that only a small part (~2mm in length) of the lesion has been found in the sliced bovine muscle (see Figure 1(a)). However, after focal correction, the lesion (~5mm in length) has been found in the sliced bovine muscle (see Figure 1(b)), which has confirmed the improvement in the targeting accuracy along the beam path. Moreover, we have also used multi-layer inhomogenous tissue sample (skin, fat, muscle) along the beam path, and it shows that the focus shift can be also corrected. In our HIFU phased array, the incident angles of all the elements are less than 40° at the interface, and the incident angles of half elements are less than 30°. Therefore, beam distortion caused by refraction and reflection has less influence on HIFU treatment of soft tissue than transcranial application.

Response_figure1_new

Figure 1. Lesion in the bovine muscle along axial axis after HIFU sonications without (a) and with (b) focal correction.

*How to determine the center of the treated region that is critical issue in this paper is unclear. Production of a very small lesion is preferred to determine the location of focus.  
High-intensity focused ultrasound (HIFU) phased arrays is becoming popular not only for ultrasound-guided system but also for MRI-guided one.*

Response: The image in the focal plane was reconstructed, and the center of each solid balls was determined by searching the circle with the highest average gray value in the large square area (see Figure 2 in the manuscript). The target was set as regular hexagon and filled with focal spots on the focal plane. The center of the target was set at the intersection of the diagonals of the square model.

*Line 40 change to "on the plane of"*

Response: “on plane of” was changed to “on the plane of ”. See line 39.

*Line 49 "picture" is not appropriate here*

Response: The phantom was scanned instead of pictured and the sentence was changed to “After sonications, the treatment plane in the phantom is scanned and the boundary of the associated lesion is extracted from the scanned image”. See line 48-49.

*Line 55 wrong use of "periodical" here*

Response: “periodical” was changed to “regular”. See line 53.

*Line 68 change "with" to "in"*

Response: “with” was changed to “in”. See line 66.

*Line 70-72 hard to understand this sentence*

Response: This sentence means that the centers of the treatment cells in the focal plane coincided with the geometric focus of HIFU phased array. In the current manuscript, the evaluation of targeting accuracy was performed in the targets with three sizes (inner, middle and outer regular hexagons), and the number of focal spots in each target varied. See line 191.

*Line 77 term of "targeting accuracy evaluation" is not good*

Response: “targeting accuracy evaluation” was changed to “evaluation of targeting accuracy” in the manuscript. See line 81.

*Line 79 change "in comparison with" to "in comparison to", thereafter*

Response: “in comparison with” has been changed to “in comparison to” throughout the manuscript.

*Line 99 this note could be deleted*

Response: The note has been deleted.

*Line 108 does slicing fresh beef require the lab coat and gloves?*

Response: “Put on the mask and rubber gloves” is for phantom preparation and has been moved to another place. See line 110.

*Line 147 Is another note of filling the cylindrical water tank with degassed water required here besides filling the water balloon*

Response: This note has been deleted.

*Line 163-169 B-mode sonographies at 0° and 90° are required*

Response: The B-mode images at 0° and 90° have been provided. See line 239 or see Figure 1.

*Line 182-183 what's the sonication time for each spot?*

Response: The sonication time for each focal spot is the same on each regular hexagon. The sonication time of 2.0 s, 2.5 s, 3.0 s were set for the focal spots located at the inner, middle, and outer hexagon, and 2.0 s for the focal spot at the geometric center of the phased array, respectively. See line 193-195.

*Line 185-186 what are the diameters of inner, middle and out circles?*

Response: The target has been changed from circles to regular hexagons. And the diagonals of the three regular hexagons are 5.4 mm, 9 mm, and 12.6 mm. See line 191-192.

*Line 188-189 hard to understand this sentence*

Response: It means that the sonication time for each focal spot on each circle is the same, and they are 2.0 s, 2.5 s, and 3.0 s for the focal spot on inner, middle and outer circles. In the current manuscript, the targets were replaced with regular hexagons of the same diameters, and the number of focal spots in each target varied. See line 193-195.

*Line 208 the term of "ablation rate" is not defined appropriate here*

Response: “ablation rate” has been replaced by “the ratio of the areas of lesion inside and outside the target to the target area”, see line 217.

*Line 238-239 how to determine the lesion sizes for each treatment cells?*

Response: The “treatment cells” has been replaced with “targets”. And the lesion sizes for each targets were determined by extracting the lesion boundaries from the scanned images through image segmentation.

*Line 247-248 it is awful to have one table in two pages, and the term of η*I *and η*O *is not defined and clear in this manuscript*

Response: The table has been put in one page, and the term of *η*I *and η*O is clearly defined in the manuscript. See line 217-218.

*Line 264-270 usually muscle is not a good ex vivo tissue samples used in the evaluation of HIFU-induced lesion because the acoustic absorption is highly dependent on the fiber orientation. Liver and kidney are more homogenous, and the produced lesions have good contrast for image processing.*

Response: We have tried using swine liver previously. However, the repetitive lesions were not obtained under the same parameters of sonication, so we did not choose the liver in the phantom. On the contrary, the repetitive lesions were formed in the bovine muscle-embedded phantom, and the lesion boundary can easily extracted from the scanned image. See Figure 3 and Table 1.

*Line 272 bad use of "induced by the buoyancy in water", maybe substitute it by "caused by"*

Response: Initially, "induced by the buoyancy in water" was substituted by "caused by". But this sentence has been deleted in the revised manuscript.

*Line 285 bad use of "over several centimeters"*

Response: “over several centimeters” was replaced with “above several centimeters in diameter”. However, this sentence was deleted after consideration.

**Reviewer #2:**

*Manuscript Summary:*

*This protocol suggests a method for a simple method to ensure targeting accuracy when using a clinical USgHIFU system with a steered phased array.*

*Major Concerns:*

*My main concern for this protocol is that it is very specific to the HIFU system used for this study, and would probably not be applicable to most other systems. This issue is further compounded by the fact that no information is given to the technical specifications of the system (i.e. how many elements, imaging frequency etc), making it even more difficult to relate to other systems..*

Response: The technical specifications of the USgHIFU phased array system have been given in the supplementary material. This protocol can be used to evaluate the targeting accuracy for the systems like ALPIUS (Alpinion Medical Systems, Seoul, Korea). For USgHIFU systems with self-focused HIFU transducer, the steps in 4.2-4.4 in PROTOCOL in the manuscript should be revised. The US image in the treatment plane can be reconstructed through the images acquired by translating the US imaging probe instead of rotation, and the other steps in PROTOCOL are the same. Therefore, the modified protocol can be used in the assessment of targeting accuracy for most USgHIFU systems with self-focused HIFU transducer such as JC HIFU system (Chongqing Haifu Technology, Chongqing, China) and HIFUNIT9000 (Shanghai A&S Science and Technology, Shanghai, China).

*Also it provided with a very simplistic approach to ensuring axial alignment, without consideration for the beampath. I.e. if a tissue path had been used, rather than a water path, could the accuracy have been measured in this way? This is more relevant to clinical applications*

Response: To mimic the beam path in the clinical settings, the protocol of our method has been modified by placing fresh swine muscle with a 30-mm thickness above the phantom. See 4.7 and 5.2 in PROTOCOL.

*Minor Concerns:*

*Section 1) - could the stl files for this holder have been provided for those with the facilities to produce, also is there a specific need for a resin printer?*

Response: The CAD drawing of the phantom holder has been provided in the supplementary material. Therefore is no need for a resin printer because the square model and the holder are rather simple. And both the square model and the holder can be used repeatedly.

*Section 2) - Please specify what 'beef' means in this context, i.e. is it muscle, liver etc?*

Response: ‘beef’ is muscle, and it has been replaced with ‘bovine muscle’ in the manuscript.

*Section 4) - For a protocol that is for checking the alignment of USgHIFU, why are there no B-mode images in these results?*

Response: The US images at the angles of 0°, 90° and the focal plane (reconstructed image) have been provided. See Figures 1 and 2 in the manuscript.

**Reviewer #3:**

*Manuscript Summary:*

*The subject manuscript describes a method to evaluate the targeting accuracy in the focal plane for ultrasound-guided phased-array high-intensity focused ultrasound system. The manuscript is presented reasonably well, but can be greatly improved. I would suggest the authors to address few of the minor comments before this can be published.*

*Minor Concerns:*

*Line 91-97; Authors need to specify the fabrication parameters,? Perhaps authors can provide the CAD drawing as supplementary material*

Response: The CAD drawing of the square model has been provided in the supplementary material.

*Line 106; Any temperature requirements?*

Response: The procedure of sticking cylindrical plastic to acrylic baseboard was performed at room temperature. And it has been added in the manuscript. See line 100.

*Line 110-112; This has to be done inside fumehood*

Response: Yes. However, due to the restrictions, we prepare the phantom near the open window and put on mask and rubber gloves as well for substitution.

*Line 117-118; Any specific reason for choosing polyacrylamide based phantoms? Why not agar or materials with substantially less safety hazards? Please specify this in the main text*

Response: Polyacrylamide based phantom is robust, stable and can be exposed to successive doses, the stiffness is larger than that of human soft tissue while the attenuation is significantly smaller than that of soft tissues. Therefore, the phantom is easier to be fixed and denatured tissue on the focal plane in the phantom can be easily identified through the optically transparent gel. And the process to make such phantom is short, usually several hours. Also safeguard such as ventilating and wear mask and rubber gloves makes the procedure little safety hazards. However, agar based phantom needs longer time to fully gel (up to several weeks), and it is soft and not easy to be fastened.

*Line 149-150; Needs to specify the temperature of water tank*

Response: The temperature of the water tank is around 22°C ~25°C, and it has been added in the manuscript See line 144.

*Line 160-161 Please specify whether the authors used an actuator, Manual or motorized?*

Response: The movement of the water tank can be realized by moving the treatment bed which is motorized in three dimension and can be both coarsely and finely controlled with buttons. And the lifting of the treatment head can be controlled by both the buttons and the user interface.

*Line 167- 169; Authors need to describe the reconstruction methodology in more detail.*

Response: In this study, the B-mode US imaging probe was rotatable, and the image in the treatment plane can be reconstructed through the image sequence acquired during probe rotation. Bilinear interpolation has been used for image reconstruction, and the detailed algorithm can be found in the previous study2. The reconstruction codes have been verified by phantom tests.

Reference

1 Li, D. H., Shen, G. F., Bai, J. F. & Chen, Y. Z. Focus shift and phase correction in soft tissues during focused ultrasound surgery. *IEEE Transactions on Biomedical Engineering.* **58** (6), 1621-1628, (2011).

2 Tong, S., Downey, D. B., Cardinal, H. N. & Fenster, A. A three-dimensional ultrasound prostate imaging system. *Ultrasound in Medicine and Biology.* **22** (6), 735-746, (1996).