

I thank the editorial team and reviewers for their thoughtful and useful comments, my response to these are in italics below. Revisions in the manuscript are highlighted in green. A separate 'clean' version was also uploaded to show the yellow highlight to provide instructions for filming production.

Editorial comments:

Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

This has been done.

2. Please define all abbreviations before use. For example: PTFE, TEMED, DAQ, TTL, OPO, HIFU, CNC, AuNR, CNC, LS, etc.

These definitions have been added.

3. Please use SI abbreviations for all units throughout: mL, μ L, min, s, etc.

This has been done.

4. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.

For example: H102, Sonic Concepts, PT3/M, Thorlabs Inc, A300, E&I, 33250A, Keysight Technologies, Precision Acoustics Ltd, MatLab, etc.

This has been done.

5. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.).

This has been done.

The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note." However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods, etc.

6. 1.1/1.2: Please label them as notes.

This has been done, L92, 95.

7. 1.3: Please add more details to how water is filtered (size of filter) and degassed. What does 60% v/v mean? Is this just water?

The term v/v or w/v are standard nomenclature indicating concentration via volume/volume percent or weight/volume percent. I have corrected this in the manuscript.

8. 1.4: Please describe the composition of these solutions or how they are prepared.

These are stock solutions from commercial suppliers. This info has been added to the manuscript, P3:L12.

9. 1.6: What is a medium stirring speed?

This is difficult to quantify with the system used, but a qualitative description has been added, L109-110.

10. 1.7: After this step, is vacuum pump turned off? In the following steps, other chemicals are added. Is stirrer kept on or off?

This has been clarified, L113-114.

11. 2.3: Please describe how this is actually done.

[Please note that 2.3 is now 3.3 in the revised manuscript]

This has been added, L125-155.

12. 2.5: Please explain how the correct connection is confirmed.

[Please note that 2.5 is now 3.5 in the revised manuscript]

This has been clarified, L179-180.

13. 2.6: How is catalyzed phantom material prepared? How long is the wait?

[Please note that 2.6 is now 3.6 in the revised manuscript]

This has been clarified, L187-189.

14. 3.2: Can the custom MatLab control program be provided as a supplemental file?

[Please note that 3.2 is now 4.2 in the revised manuscript]

Yes, it will be uploaded with the revised manuscript.

15. 3.4: Please specify the pressures used.

[Please note that 3.4 is now 4.4 in the revised manuscript]

This has been added, L236-237.

16. Please ensure that only protocol steps with actionable items are highlighted for inclusion in the video. Please ensure that all highlighted steps can clearly be visualized. For example, steps 3.6 and 4.5 should not be highlighted for inclusion in the video.

[Please note that 3.6 and 4.5 are now 4.4 and 5.5 in the revised manuscript]

This has been revised.

17. Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense.

This has been checked.

18. Representative Results: Please do not number each sentence.

This has been revised.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

The manuscript describes a combination of nanoparticles, pulsed laser and HIFU for promoting cavitation occurrence in a tissue mimicking phantom. It is essentially a carbon-copy of the Phys Med Biol paper by the same author (ref 6). I imagine a JOVE format publication could be useful for other researchers in the field.

Major Concerns:

None

Minor Concerns:

I think the manuscript would benefit from further discussion around the challenges/limitations of translating the technique toward clinical application (laser penetration into tissue, nanoparticle delivery etc)

A short section in the discussion has been added to address this, L344-350.

Reviewer #2:

Manuscript Summary:

The manuscript provides benchtop protocols that describe methods to fabricate tissue-mimicking phantoms and to quantitatively investigate enhancement in inertial cavitation and associated heating during hyperthermic HIFU exposures. Overall, this manuscript is well written and could potentially provide users interested in noninvasive hyperthermic treatments with an interesting set of bench-top protocols for optimizing light-absorbing nanoparticles as well as the acoustical/optical parameters for enhancing ultrasound-mediated heating.

Major Concerns:

But the manuscript is lacking meaningful comparisons in heating or thermal damage between regular HIFU application and HIFU with laser-aided nucleation of inertial cavitation. This is a critical omission that significantly diminishes the enthusiasm for this manuscript. Furthermore, the purpose of the protocol is not well motivated. I guess it could be that when presented with various light-absorbing nanoparticles, one might be interested in determining the optimal acoustical and optical parameters to maximize inertial cavitation and cavitation-enhanced ultrasonic heating, or one might want to use this benchtop protocol to identify the most efficacious nanoparticle agent for enhance HIFU therapy, but this is not clearly stated in the manuscript.

Thank you for your suggestion. A short discussion regarding the thermal damage has been added to the manuscript L357-363. As with the previous reviewers' comments, further motivation regarding clinical translation has been added L344-350.

Minor Concerns:

Title: Controllable nucleation of cavitation for enhancing ...

The title does not seem representative of the research objective. The intent is to enhance ultrasound-mediated heating, not enhance HIFU exposures

This is been corrected, the new title reads 'Controllable nucleation of cavitation from plasmonic gold nanoparticles for enhancing high intensity focused ultrasound applications'

Line 53-57: please rephrase; this is confusing and effective

This has been revised, L54-58.

Line 61: typo - these can take for the ...

This has been corrected, L64.

Line 69: rephrase, poorly structured sentence

This has been revised, L69-72.

Line 85: The protocols presented do not demonstrate improved treatment guidance or increase in thermal ablation. They merely describe methodology for demonstrating an increase in inertial cavitation - and maybe, the feasibility of laser-enhanced ultrasonic heating, where the underlying physical mechanism is laser-induced nucleation for increasing the propensity for inertial cavitation.

This has been revised.

Line 107: medium stirring speed is very vague.

This has been clarified, L108-109.

Line 110: What vacuum pressure is adequate?

The vacuum pressure has been added, L112.

Please provide specific geometric parameters for the two transducers. This will help explain the 85 microsecond value.

This has been added.

Line 139: Is the broadband hydrophone referred to in this text the same as the PCD? *In this study PCD, is defined as passive cavitation detection, and thus mostly used in context of PCD system, which the hydrophone is a part of. I ask acknowledge that PCD is often also called passive cavitation detector. However, in this protocol the broadband hydrophone is a component of the PCD system.*

Line 167: step 2.9: The laser alignment procedure seems very imprecise. Why not color the ball bearing black and use its photoacoustic signal to precisely align the PCD with the ball bearing?

This is a good suggestion. However, due to space limitations and need to keep the fibre optic outside of the acoustic field, the peak photoacoustic emission (the ball bearing alone generates sufficient emission to detect), does not correspond to the same location as peak acoustic reflection. Furthermore, precise targeting of the laser beam is not needed as the beamwidth is 15 mm, which is larger than the focal peak of the HIFU system. For in vivo or clinical applications the light would become diffusive after about 1 m penetration into tissue, so it would not be possible to precisely target a region.

Line 174: What is the field of view and working distance for the microscope at the magnification used for monitoring the thermal lesions?

This has been added to section 3.10.

Line 189: Why is the PCD disconnected? This is not clear?

This comes back to the early definition of PCD. For this part of the alignment procedure the hydrophone was connected directly to the DAQ card to visualise the pulse-echo signals for alignment. The high pass filter would have removed the fundamental 3.3 MHz, which was being used for alignment. The 40 dB pre-amp is also not needed as sufficient backscatter off the metallic target was necessary for detection.

Please include labels on the figure that show the peak negative pressure and fluence used in each panel for facilitating comparison.

These have been added to Figure 4.

Figure 3 does not seem to contribute much here. If they had shown differences in lesion with and without laser-aided nucleation, that would have been more insightful.

Thank you for the suggestion. This figure has now been revised to include a time series for with/without the laser and nanoparticles.