Reviewer Comments and Author Response for:
JoVE 62433

We have found all of the comments provided by the reviewers to be very helpful in improving and clarifying the revised manuscript. We are grateful for the time and effort by the reviewers in providing feedback, and have addressed each comment individually. A summary of the manuscript changes and addressed reviewer comments is provided below.

Response to Editorial Comments:

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

***Response**: A proofread for spelling and grammar issues was done.*

Editor: Please include a Summary to clearly describe the protocol and its applications in complete sentences between 10-50 words: "Presented here is a protocol …"

***Response:** A summary section was added at the beginning of the manuscript (after the keywords section).*

Editor: Please ensure that the Abstract is between 150-300 words.

***Response:** Abstract was reviewed to ensure that includes 150-300 words.*

Editor: For in-text formatting, corresponding reference numbers should appear as numbered superscripts after the appropriate statement(s).

Response: Reference numbers were changed to superscript.

Editor: JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. 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.

***Response:** All commercial language and references were removed from the manuscript.*

Editor: Line 66: Please change proposed protocol to Protocol instead.

***Response:** Name of the section was changed to "Protocol".*

Editor: We cannot have non-numbered paragraphs/steps/headings/subheadings.

Response: *All non-numbered paragraphs/step/heading/subheading were numbered or added to another section. Paragraph on line 123 was added to the step 2.3.10. Paragraph on line 138 was added to step 3.4.*

Editor: Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed?

Response: *More details were added to steps.*

For step 1.1, details about where the sample were obtained, the dimensions, and machine used to cut the sample, were added.

For step 1.1.3, details of how the polishing was made was added.

For step 2.1, "sonicate" was changed to "using an ultrasonic cleaner" for clarity.

For the tensile test section, parameters such as maximum displacement, returning rate and we execute the micro-tensile test (performing a negative displacement indentation), were added.

Editor: Please include all details associated with a step in complete sentences.

Response: *All steps were changed to complete sentences.*

Editor: How big is the sample- dimensions? How much sample is cut? What do you use for polishing?

Response: *Figure 1 was added to explain from where the sample was obtained from and its dimensions. Also, a suggested material sample thickness of 6 mm was added. In the step 1.1.3 "using a semi-automatic polisher" was added.*

Editor: 2.1: Volume of solutions used? How do you perform sonication? Do you do this on ice? How do you take care of heating?

Response: *The "sonicate" word was changed to "using an ultrasonic cleaner" for clarity. Also, there is not specific volume, so, it was specified that the solvent just need to cover the sample.*

Editor: 7: How do you perform tensile testing?

Response: *Step 6: Micro-tensile test, contains all the steps and recommendation that we could provide to perform the testing. Since specific procedure may depend on the machine used, not a lot of details can be provided. However, test parameters such as displacement rate, maximum displacement, returning rate, and how we did the test (performing a negative displacement indentation and using a negative displacement rate) were added (see section 6.6) to provide added detail.*

Editor: Please ensure the numbering of the Protocol follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary. Please refrain from using bullets, alphabets, or dashes. Please do not go beyond third substep.

Response: *Protocol was revised and all steps that were beyond the third sub-step were formatted. Spin coating parameters, which were in a fourth sub-step, were formatted in a table.*

Editor: Please remove the embedded figure(s) and tables from the manuscript. All figures should be uploaded separately to your Editorial Manager account and all tables must be uploaded as .xlsx file. Each figure/table must be accompanied by a title and a description after the Representative Results of the manuscript text.

Response: The tables and Figures have been removed from the manuscript for individual upload.

Editor: Please include a single line space between each step and substep and highlight 3 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.

Response: A space has been added between each step/sub-step as suggested, and the essential steps for the video have been highlighted in green in the manuscript.

Editor: Please describe the result with respect to your experiment, you performed an experiment, how did it help you to conclude what you wanted to and how is it in line with the title.

Response: *Language was added and the Representative Result section was modified to better describe the experiment and result within the context of the procedures presented. The section now begins by stating:*

"A material sample from an AM 17-4 PH stainless steel specimen (previously tested in low-cycle fatigue) was prepared and tested using the protocol described, to understand the fundamental material behavior of AM metals (independent of structural defect influence). Typical sample volumes used for material characterization can contain distributed fabrication/structural defects that make discerning between actual material behavior and structural fabrication effects difficult. Following the protocol described in Sections 2 through 7, a micro specimen was fabricated and tested to failure in tension, successfully demonstrating the described techniques and producing material test data at scales free from volumetric defect influences."

Editor: We do not have conclusion section. Please include a discussion section instead. Please ensure that the Discussion explicitly cover the following in detail in 3-6 paragraphs with citations:
a) Critical steps within the protocol
b) Any modifications and troubleshooting of the technique

c) Any limitations of the technique
d) The significance with respect to existing methods
e) Any future applications of the technique

Response: *The previous conclusions section has been re-named "Discussion" and various aspects of developed procedures (including troubleshooting, limitations, significance, etc.) have been added.*

Editor: Please sort the Materials table in alphabetical order.

Response: *The materials table has been sorted in alphabetical order as suggested.*

Editor: Please include an Acknowledgements section, containing any acknowledgments and all funding sources for this work.

Response: *An acknowledgement section was added.*

Editor: Please include a Disclosures section, providing information regarding the authors' competing financial interests or other conflicts of interest. If authors have no competing financial interests, then a statement indicating no competing financial interests must be included.

Response: *A disclosures section was added.*

Response to Comments from Reviewer #1:

Reviewer #1: Could the authors provide the surface roughness of the sample after polishing?

Response: *This is a good suggestion that deserves further clarity in the manuscript protocol description. Section 1.1.3 now has the expected surface roughness described from the polishing step:*

"Starting from 400 grit abrasive paper and moving to 1 µm diamond particles, polish the sample to mirror-like surface (having a surface roughness on the order of 1 µm), using a semi-automatic polisher."

Reviewer #1: What is the thickness of the SU-8 photoresist? Why did the authors choose SU-8? Is it possible to use a positive photoresist?

Response: *The thickness of the SU-8 photoresist was measured to be near 1.5 microns on average. The choice to use the SU-8 was based on lab availability and its robustness. Other photoresist and etching combinations could also be effective; however, the combination presented in the manuscript was found to be the most effective for our purposes. The following discussion was added to the "discussion" section of the manuscript to clarify these points:*

*"During the development of this process, variation within the photo-resist mask patterning was noticed, as shown in **Figure 2**. This is likely caused by surface inconsistencies created during dicing or poor adhesion of the photoresist to the sample surface. It was noticed that when wet etching was performed at room temperature, much of the photoresist was removed, due to under etching or poor adhesion; therefore, it is recommended to warm the sample before and during the etching process, as mentioned in the protocol. If significant under-etching (etching below the photoresist) is noticed, increasing the sample temperature may help. The provided protocol uses an SU-8 photoresist due to availability; however, other photoresist and etchant combinations may also be effective."*

The following phrase was added at the end of section 2.3.3:

"Note that the thickness of the resulting SU-8 photoresist used in this study was measured to be near 1.5 microns on average."

Reviewer #1: The authors suggested that some SU-8 microstructures were detached from the surface of the sample because of the uneven surface (Figure 1). Does this happen often? I suspect that it was because of the poor adhesion between SU-8 and the sample.

***Response**: This is a good point. We originally thought that the detachment was only due to un-even surface because we saw that after the spin coat there were spots with no photoresist. Also, SU-8 have been used previously in other steels in the lab with no problem. However, we noticed that some of the patterned photoresist were washing out during the etching process. Although some detachment of photoresist was seen, the result that were getting was good enough for the purpose. A text was added in the discussion section, to talk about this issue.*

"*During the development of this process, it was noticed that the pattern was not always perfect, as shown in **Error! Reference source not found.**. This can be attribute to a possible lack of surface level caused by the dicing process, allowing a non-uniform photoresist distribution on the surface. Also, this could be due a possible poor adhesion to the sample. It was noticed that when wet etching was performed at room temperature, most of the photoresist washed out, due to under etching or poor adhesion. Therefore, it is recommended to warm the sample before and during the etching process, as mentioned in the protocol.*

This protocol uses SU-8 due to availability and because it is well known to be robust. However, this does not mean that another photoresist – etchant combination cannot be explored. Although some challenging were faced during the photolithography and wet-etching process, the result were good enough for this purpose."

Response to Comments from Reviewer #2:

Reviewer #2 I'm interested to see a more complete data set if you intend to complete additional tests. Modeling could be useful in corrections for alignment/orientation which you acknowledge is a particularly challenging aspect of these tests.:

***Response**: We are grateful for your comments and interest in our project. We are currently working on developing a robust material behavior database from these tests; however, the scope of the current manuscript was limited to providing a proof of concept methodology for fabricating and performing the micro-tensile tests on AM17-4PH steels. We plan on publishing further works related to the microscale material behavior and fracture prediction upscaling models.*

Response to Comments from Reviewer #3:

Reviewer #3: I found the paper interesting and well-developed. The description of the protocol is exhaustive and detailed. The protocol efficacy has scientific soundness. Further, its efficacy has been achieved and supported by sufficient data.
Best Regards

***Response**: We appreciate your comment and the time that you dedicated to review our work.*