J1705-12345-Doe_FS
Report prepared by: Steve Jones, PhD

**Manuscript Summary**

| Title: | Full manuscript title |

**About Your Expert Scientific Review Report**

Key

✔️ No changes necessary
✔️ Minor revisions suggested; see the comments for more information
❌ Major or important revisions suggested; see the comments for more information

Please note that this report focuses on the content of your manuscript rather than the language. If you have ordered English language editing, please send your revised manuscript to us for editing.
### Title and Abstract

| The title is appropriate and indicates the main message of the paper | ✓ |
| No changes necessary. | |
| The abstract provides a concise but complete summary of the content of the paper | ✗ |
| The present abstract does not explain why this study was performed. | |
  - Please clarify the gaps in the existing literature and highlight the problem that this study addresses. The first paragraph of the Results and Discussion section summarizes these points; please include this information in your abstract. |
| The abstract can stand alone without reference to the main text (e.g., abbreviations and jargon clearly defined) | ✗ |
| The abbreviation ASM is used, but it is not defined and readers may not be familiar with this term. | |
  - Please define “ASM” and provide a brief description of its purpose. |
| The abstract is understandable by a non-specialist reader | ✓ |
| No changes necessary. | |

### Introduction

| The introduction provides sufficient background information for readers not in the immediate field to understand the problem/hypotheses | ✓ |
| The introduction would benefit from the addition of more information about the current applications of related technology. | |
  - Please briefly describe and cite recent studies that have applied related technology for similar purposes. |
| The reasons for performing the study clearly defined (e.g., the gap in the existing knowledge and the importance of the topic are clearly defined) | ✗ |
| The connection between the requirements of high resolution and accuracy for metrology applications, and the necessity of choosing an appropriate reconstruction technique is not made clear. Additionally, although the importance of selecting an appropriate reconstruction technique is emphasized, the reason for selecting ASM for image reconstruction in this work is not explained. | |
  - Please explicitly state that inappropriate reconstruction techniques can lead to inferior results, and cite supporting references. |
  - Please explain your reasons for selecting ASM for this work (in particular, you could emphasize its benefits for sectional image reconstruction). |
| The study objectives are clearly and explicitly defined | ✓ |
No changes necessary.

The objectives match the results and conclusions  

No changes necessary.

## Methods/Technical Rigor

The methods used are appropriate to the aims of the study  

No changes necessary.

Sufficient information is provided for a capable researcher to reproduce the experiments described  

Some of the method descriptions need additional information

- Please provide more information about the spatial filter.
- It appears that no scanning was carried out—if this was the case, please state this explicitly.

No additional experiments are required to validate the results of those that were performed  

Although this is not absolutely necessary, the paper would be improved if you could demonstrate that the results obtained using the present method are consistent with results obtained using a different technique.

- Please consider repeating the experiment using the current standard technique for comparison.

There are no additional experiments that would greatly improve the quality of this paper  

The advantages of the numerical filtering, and therefore of the sectional reconstruction technique, are not made completely clear by the range of experiments currently presented.

- If possible, please show images at the focus plane with and without the out-of-focus elements (that is, with and without the numerical filtering). This may make the advantages of the numerical filtering more obvious.

Appropriate references are cited where previously established methods are used  

No changes necessary.

## Results/Statistics

The results are clearly explained and presented in an appropriate format  

The ASM is explained in other papers, and the aim of the present paper is not to develop a novel reconstruction technique. I therefore feel that too much space is used explaining the ASM.

- Please consider reducing the explanation of the ASM in Section 2, and instead, provide a more in-depth discussion of the sectional images shown in Section 3.

The figures and tables show essential data that could not be easily summarized in the text  

Figure 3 does not appear to be vital to the discussion presented in the paper. It is only cited in two sentences, and in neither of those are the results that it presents discussed.

- Please consider removing Figure 3 and focusing the discussion on Figures 4 and 5, which are more relevant to the topic of the paper (sectional reconstruction).

<table>
<thead>
<tr>
<th>Aspect</th>
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<tr>
<td>There is no unnecessary duplication of data in the graphics and/or the text</td>
<td>✓</td>
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<tr>
<td>No data are duplicated in the graphics and/or text. However, Figure 3 does not seem essential to the paper.</td>
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<tr>
<td>Please consider removing Figure 3.</td>
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<tr>
<td>The figures and tables are easy to interpret</td>
<td>✓</td>
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<td>The figures and tables are mostly easy to interpret. However, two panels in Fig. 4 have the same labels (d). Additionally, the z coordinate is not stated on Figure 2.</td>
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<td>Please check and amend the labels in Figure 4.</td>
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<td>Please add the z coordinate to Figure 2.</td>
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<td>There are no additional graphics that would add clarity to the text</td>
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<td>No changes necessary.</td>
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</table>

No data are duplicated in the graphics and/or text. However, Figure 3 does not seem essential to the paper.

- Please consider removing Figure 3.

There are no additional graphics that would add clarity to the text

- No changes necessary.

Appropriate statistical methods been used to test the significance of the results

- This is not relevant to the present paper.

P-values and other indicators of statistical significance are included where necessary

- This is not relevant to the present paper.

**Discussion**

All possible interpretations of the data considered; there are no alternative hypotheses that are consistent with the available data

- This is not relevant to the present paper.

Figures 4 and 5 are not discussed in depth. It is not clear why the 3D perspective is insufficient for analysis, and it is not clearly demonstrated by the data presented that the resolution obtained with this technique in the focus plane is sufficient for analysis. Moreover, the practical benefits of the improved imaging results are not clearly discussed.

- Please discuss why the 3D perspective is insufficient for analysis.
- Please highlight your findings that demonstrate that the resolution in the focus plane is sufficient for analysis.
- Please discuss the practical benefits of the improved imaging; for example, discuss what biologically relevant information can be obtained from the in-focus image that cannot be obtained from the out-of-focus image.

The findings of the study are properly described in the context of the published literature

- This is not relevant to the present paper.
The published literature is presented as background information and is not connected to the specific findings of this study. In particular, the benefits of imaging small particle fields is not made clear, and the choice of specimens (and how this compares with those used in previous studies) is not discussed.

- Please directly compare the approach that you used in this study with those used in previous studies. In particular, please comment on how the results compare and how this might have been affected by differences in the specimens used.

The limitations of the study are discussed

The limitations of the study are not discussed.

- Please mention the tradeoffs involved in selecting ASM over current standard methods.

The conclusions of the study are supported by appropriate evidence; the claims are not exaggerated

The conclusion says that the digital holographic method has potential biomedical imaging applications in 3D microscopy; however, the discussion of Figs. 4 and 5 does not make this point obvious.

- If you wish to make this claim, please consider providing a more in-depth discussion of this point in the discussion of Figures 4 and 5.

The conclusions address the aim of the study

No changes necessary.

**Literature Cited**

The literature cited is appropriate, recent and balanced

No changes necessary.

There are no statements that are missing citations of have an insufficient number of citations, given the strength of the claim made.

No changes necessary.

**Significance and Novelty**

The claims are sufficiently novel to warrant publication

No changes necessary.

The study presents a conceptual advance over previously published work

The study uses concepts previously used in different but related contexts in the literature.

The study presents an incremental advance over previously published work
The digital holographic image reconstruction technique presented in this paper builds on previous work. The simplicity and power of the technique presented represent an incremental advance over existing techniques.

**Journal Selection**

The target journal (if known) is appropriate

The scope of the target journal, *Applied Optics*, is appropriate for the topic of the paper. However, in its current form, the study may not be considered novel or important enough for publication in this journal.

- *If you wish to publish in Applied Optics, please consider providing a more detailed analysis of Figures 4 and 5, and more clearly discussing the practical consequences of the improved resolution.*
- *If you do not want to make this change, or cannot, please consider selecting a target journal with a lower impact factor.*

The likely target audience of this paper includes researchers interested in application of holography (as opposed to researchers doing fundamental research in optics). This will likely include researchers in diverse fields (including biology, materials science, nanotechnology, physics, mechanical engineering, and image processing) who are looking for techniques that will facilitate their research.

**Summary**

**Major action points**

- State your rationale in both your abstract and your introduction.
- Extend the discussion of Figures 4 and 5 in the context of the existing literature.
- Discuss the limitations of the technique.
- Consider repeating the experiments with the current standard technique for comparison.

**Minor action points**

- Define and briefly describe “ASM” in your abstract.
- Provide more background on current applications of related technology in the introduction.
- Provide more information about the spatial filter in the methods.
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