

**Introduction**

**Does the introduction provide sufficient background information for readers not in the immediate field to understand the problem/hypotheses?**

The introduction provides a good, generalized background of the topic that quickly gives the reader an appreciation of the wide range of applications for this technology. However, to make the introduction more substantial, the author may wish to provide several references to substantiate the claim made in the first sentence (that is, provide references to other groups who do or have done research in this area). The second sentence helpfully explains the motivation for the research to current and potential funding agencies. However, to make the motivation clearer and to differentiate the paper some more from other applied papers, the author may wish to provide another sentence giving examples of some of the applications of this technology, along with appropriate references.

**Are the reasons for performing the study clearly defined?**

I think the motivations for this study need to be made clearer. In particular, the connection between (a) the requirements of high resolution and accuracy for metrology applications, and (b) the necessity of choosing an appropriate reconstruction technique, could be clearer. One way to demonstrate this connection would be to cite references (if possible) that demonstrate that inappropriate reconstruction techniques can lead to inferior results.

Furthermore, after stating that the choice of reconstruction technique is important, the author offers no explanation of why he chooses the ASM for image reconstruction in the present work. I think the motivation for the present research would be clearer if the author could provide a more direct link between the importance of choosing an appropriate reconstruction method and sectional image reconstruction.

<b>Are the study objectives clearly defined?</b>	The objective is clearly defined in the last sentence of the second paragraph. However, I feel this sentence could be modified to something like “In this publication, we show that the selective numerical reconstruction method is advantageous in 3D microscopy and tomographic imaging using digital holography.”
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**Methods/technical rigor**

<b>Are the methods used appropriate to the aims of the study?</b>	The experimental apparatus is quite standard, and is appropriate for the study, especially given that the main focus of the paper is not to develop a novel holographic technique, but to demonstrate the power of sectional image reconstruction.
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<b>Is sufficient information provided for a capable researcher to reproduce the experiments described?</b>	Yes, although the author should probably provide more information about the spatial filter. Also, the author may wish to mention in this section the advantage that no scanning is necessary with this method (as opposed to scanning optical holography).
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<b>Are any additional experiments required to validate the results of those that were performed?</b>	I don't think any additional experiments are necessary to validate the results presented here, because the results themselves are not what is important; it is the technique used to obtain these results that is important. One exception to this reasoning would be if the author could demonstrate that the results obtained using the present method are consistent with results obtained using a different technique. I don't think this is vital to the present paper (especially given the length limitations on the paper), but it may be something that would be helpful in a longer, more detailed paper.
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<b>Are there any additional experiments that would greatly improve the quality of this paper?</b>	To clearly show the advantages of the sectional reconstruction technique, would it be possible to 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.
<b>Are appropriate references cited where previously established methods are used?</b>	There are several instances where assertions are made that are not substantiated with references. These have been noted in the appropriate sections of this report.

## Results/statistics

<b>Are the results clearly explained and presented in an appropriate format?</b>	Because the ASM is explained in other papers, and because the aim of the present paper is not to develop a novel reconstruction technique, the author may wish to reduce the explanation of the ASM in Section 2, and instead, provide a more in-depth discussion of the sectional images shown in Section 3.
<b>Do the figures and tables show essential data or are there any that could easily be summarized in the text?</b>	It seems to me that Figure 3 is not 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. I would suggest removing it and focusing the discussion on Figs. 4 and 5, which seem to me to be more relevant to the topic of the paper (sectional reconstruction).
<b>Are any of the data duplicated in the graphics and/or text?</b>	No data is duplicated, but the data in Fig. 3 does not seem vital to the paper.
<b>Are the figures and tables easy to interpret?</b>	Two panels in Fig. 4 have the same labels (d). Presumably this is an error. Also, it may be helpful to the reader to show the coordinate $z$ on Fig. 2.
<b>Are there any additional graphics that would add clarity to the text?</b>	I do not think any additional graphics are necessary. However, as noted above, I think Fig. 3 is unnecessary and could be removed, which would allow a more in-depth discussion of Figs. 4 and 5.
<b>Have appropriate statistical methods been</b>	This is not relevant for the present paper.

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used to test the significance of the results?

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## Discussion

**Are all possible interpretations of the data considered or are there alternative hypotheses that are consistent with the available data?**

As suggested above, I think a more in-depth discussion of Figs. 4 and 5 would be helpful. I feel this is an important result for this paper, and therefore it merits more discussion. Why is the 3D perspective insufficient for analysis? Can the author demonstrate that the resolution of certain small particles is less than required for a given application? More importantly; can the author show that the resolution in the focus plane is sufficient for analysis (which is not obvious from the images)?

The author may also wish to give a more detailed discussion of Fig. 5. Can the author show an out-of-focus plane and 3D image for this specimen (this image is easier to interpret than the images of small particles, for which it is hard to differentiate by eye the difference between focused and out-of-focus planes)? Can the author demonstrate what biologically relevant information he can get from the in-focus image that is not possible to get from the out-of-focus image?

**Are the findings properly described in the context of the published literature?**

The author may wish to mention why it is important to image small particle fields to explain the motivation for his choice of specimens, and accompany this with some references to other studies that demonstrate this importance.

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**Are the limitations of the study discussed? If not, what are the major limitations that should be discussed?**

No significant limitations are discussed. It may be worthwhile to mention the tradeoffs involved in choosing the ASM as opposed to some other method. This may be done in Section 2 after describing the advantages of ASM.

## Conclusions

**Are the conclusions of the study supported by appropriate evidence or are the claims 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. This conclusion would be much stronger were such a discussion provided (see Discussion section, above).

## Literature cited (introduction, results, discussion)

**Is the literature cited balanced or are there important studies not cited, or other studies disproportionately cited?**

The literature cited is relevant to the study, but there are several instances, which have been noted above, in which the author makes assertions without substantiating them with references.

**Please identify statements that are missing any citations, or that have an insufficient number of citations, given the strength of the claim made.**

I have noted these in the sections above (see, in particular, the section on the introduction).

## Significance and Novelty

<b>Are the claims in the paper sufficiently novel to warrant publication?</b>	As it stands, I am not sure that the results will be judged novel or important enough for publication in <i>Applied Optics</i> . However, if the author provides a more detailed analysis, in particular of Figs. 4 and 5, I think the paper could prove to be very interesting and useful to a very large audience, possibly making it acceptable for publication in <i>Applied Optics</i> .
<b>Does the study represent a conceptual advance over previously published work?</b>	The paper provides an excellent technique for digital holographic image reconstruction because the technique is relatively simple to implement and yet it is quite powerful. Also, being simple, it can be reproduced by many who are not necessarily specialists in optics.

## Journal Selection

<b>Is the target journal (if known) appropriate? If not, why not?</b>	The four major topics covered by <i>Applied Optics</i> (the target journal) are optical technology, information processing, photonic devices, and biomedical optics. The manuscript deals with digital reconstruction of holographic images, which is a technique that can be applied to biological samples and to metrology. As such, the manuscript's topic relates to the first two and the last of the major topics of <i>Applied Optics</i> (and one could also argue that it relates to the third topic). Therefore, I believe the target journal is an appropriate forum for this article. In addition, <i>Applied Optics</i> is a popular and respected journal, so publication in this journal will ensure a wide readership and good exposure for the author's work.
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<b>What is the likely target audience of this paper? Please comment on the specific field (e.g., diabetes, neurology) and activity (e.g.,</b>	Because the article discusses a method to perfect digital holographic image reconstruction, I expect it will draw interest from researchers who want to apply holography to their research (as opposed to researchers doing fundamental research in optics). Therefore, I expect that the likely audience for this
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clinician, researcher).

article will be researchers in diverse fields (including biology, materials science, nanotechnology, physics, mechanical engineering, image processing...) who are looking for enabling techniques that will allow them to progress their research.

### Minor comments

Please refer to the comments in the edited manuscript file for minor comments.

### Major comments

**To publish this paper in your target journal, the following revisions are strongly advised:**

- Regarding the figures: I recommend removing Fig. 3, shortening Section 2, and devoting the additional space to a more detailed discussion of Figs. 4 and 5. In particular, the author may wish to point out in-focus and out-of-focus spots in Fig. 4 (e.g., with arrows or labels), and to provide a more thorough analysis of these features. As it stands, only the out-of-focus spots in Fig 4(a) have earned a mention in text. It may be helpful (to show the focusing properties) to show a 2D cross section of one or several spots from Figs. 4(a), 4(c), and 4(d), so the reader can appreciate why the choice of image reconstruction plane is important.
- I recommend strengthening and clarifying the introduction, as detailed above.
- Because the author mentions in the abstract (and especially in the last, or concluding, sentence) that the object-to-hologram distance can be quite small, I expected this to be a major theme in the paper. However, very little mention of this phenomenon is made. If this is a significant advantage of this holographic imaging technique, it should be discussed in more detail in the paper. It could be mentioned, for example, in Section 3.