Response to Reviewer Comments:

**We warmly thank the reviewers for valuable comments, and have addressed any issues on a point by point basis in the following summary. We are heartened that the reviews have strengthened the manuscript.**

**Best Regards,**

**WML and Co-Authors**

Reviewer #4: *Summary:*

The authors proposed the use of 3 different 3D printing techniques to create models out of pre-clinical x-ray images. Although the manuscript presented something of interest, the concerns listed below should be adequately addressed.

*Major Concerns:*

Stronger justifications are required for people to print a 3D model using images obtained from a preclinical x-ray machine. What are the real advantages here? How can we justify this type of practice? Can we do the same thing with other imaging modalities? In terms of education, perhaps one can consider building a 3D model for learning about anatomy (with removable organ parts) just like the organic chemistry model kit. For the purpose of research, perhaps printing a mold for each individual rodent for repeated scans in a longitudinal study or a multi modality study (e.g. assessment of efficacy of drug treatment or radiotherapy).

**We agree with the reviewer, and have added the following two sentences to strengthen the long abstract:**

**“The real benefits of this method result from the tangible experience a researcher can have with data that cannot be adequately conveyed through a computer screen. The translation of pre-clinical 3D data to a physical object that is an *exact* copy of the test subject is a powerful tool for visualization and communication, especially for relating imaging research to students, or those in other fields.”**

*Minor Concerns:*

1. p.4 (Open original MicroPET file): The manuscript is about microCT studies, so is MicroPET the file name given by Carestream? It is odd to name a program used in a multi-modal scanner this way.

**MicroPET is the name conventionally given to image files acquired by the Albira Imaging System. We have updated the text under 3.1 as follows:**

**“Open original MicroPET (data format for all modalities on the Albira Imaging System) file”**

2. p. 4, last line: -1000 must be HU (Hounsfield Units). Please include this and explain what HU is.

**The text under 3.3 has been updated as follows:**

**Under the ‘VOI Tools’ tab select ‘Mask Outside Selected VOI’ and set the masking value to -1000 Hounsfield Units (HU, radiodensity scale for CT), which will effectively set the external space to the CT density value of air.**

3. p. 5 (save as Analyze file): Analyze was not mentioned as one of those programs used in the manuscript. Why the data were saved as an Analyze format?

**Analyze files were mentioned earlier in the segmentation section. To avoid confusion, segmentation and converting to DICOM have been split into two sections, making it necessary for the lung to be saved as an Analyze file in between sections.**

4. p. 6: Explain the differences between Meshlab and Netfabbb Studio Basic?

**Section 3.4 has been updated as follows:**

**“3.4 Two programs, Meshlab v1.3.1 and Netfabb Studio Basic 4.9, will concurrently remove any excess mesh, join together disconnected meshes, repair holes, and smooth the final mesh. The primary differences between these two programs are the tool sets made available to the user, and some of the interface navigation control. They are both 3D mesh editing software programs, and their use together provides the easiest approach to editing the model.”**

5. p. 11 (Figure 1): There were differences in the 3D printed models using the 3 different methods. For example, some bone segments are shorter in one model vs another model. The neck portion was missing using Shapeways Inc. The model made by the Marker Replicator shows a green object on the neck. Explanation on these types of observations are needed.

**The caption of Figure 1 has been updated as follows to improve clarity:**

**CAPTION: Figure 1: 3D printed models of the lungs and skeletal features of a rat X-ray CT data set. Objects were printed using a ProJet HD 3000 (left), Shapeways Inc. (Center) or a Makerbot Replicator (right). The scale bar denotes 2 cm. Note that the scale bar in panel C is smaller than that of A and B, which reflects that in some cases the Makerbot must print an enlarged object in order to output sufficient detail.**

6. p. 12 (Table 1): It appears that Shapeways provides a reasonable choice for most labs. How would a turn-around time of two weeks impact negatively on an operation, especially for an educational institution? How frequently should a lab use this type of 3D printing?

**The pros and cons of each type of printing have been detailed in Table 1 and accompanying text. Given the methods format of this manuscript, we will humbly leave it to the researcher’s discretion as to which strategy is most ideal, and how the lag-time, print quality, and cost will affect their individual objectives.**

7. p. 13 (conclusion): 3D rendering displayed on a nice monitor can provide a lot of useful information in 3D. How much value would 3D printing add to this?

**The following sentences have been added to strengthen the conclusion:**

**“Although representation on 2D screens has come a long way, there is absolutely no replacement for the visual and sensory experience of holding a real object that is able to be held, rotated, examined and moved around. A model paired with an electronic data representation is even more powerful as it allows researchers to examine the physical object for regions of interest, and to find those areas on a computer model for further quantitative analysis.”**

**“Finally, we provide detailed instructions to enable three-dimensional model printing with a range of commercial solutions. In each case, the end result is a model that provides a unique, hand-held, physical manifestation of the acquired tomographic data that would normally be restricted to a computer screen.”**

8. p. 14 (Required Programs): What was ReplicatorG used for?

**In section 4.1 the manuscript discusses how ReplicatorG is used to communicate with the Makerbot.**