Reviewers' comments:
Reviewer #1:
Manuscript Summary:
The manuscript by Hirsinger and Steventon provides a useful method for mounting zebrafish embryos to image the process of axial elongation. This period of development has proven difficult to image over long periods of time as the embryo undergoes dramatic morphological changes that make it difficult visualize using standard methods. They provide a variation for using a light sheet microscope. Additionally, this method has potential for use with other vertebrate and invertebrate embryos. The data presented clearly show that this is an effective method for imaging this period of development. I have some very minor comments on the manuscript.

Major Concerns:
None

Minor Concerns:
1. Typo on line 74 "agarose is enables"
- This has been corrected as highlighted in the text
2. Line 97, "1% agarose," since the method so far has used low melt agarose, it would be useful to clarify here that this is standard melting temperature agarose.
- This has been corrected as highlighted in the text
3. Lines 99-100. Is it essential to use the capillary described here? Different labs may have different capillaries on hand and so some comment about whether similar but different capillaries would be suitable would be helpful. For instance, is it critical to have the filament in the capillary?
- We have added in the notes a statement that makes it clear that filament capillaries are not a specific requirement
4. Typo line 168 "same imagine dish"
- This has been corrected as highlighted in the text
5. A catalog number for the Mattek glass bottom dishes would be helpful.
- This has now been added to the equipment list

Additional Comments to Authors:
None

Reviewer #2:
Manuscript Summary:
Zebrafish is an excellent system for imaging, and the method described here will be of broad use. I am certain it will be widely used as long as it is very clear. As a test I gave this to my very competent lab manager to try to look for problems. Her comments are below. Obviously we were handicapped by not having the instructional video, but she couldn't get the procedure to work. Specifically when she tried to put the specified amount of agarose on the microwell it didn't stay contained in the well, and this has prevented her from going further with the approach. We can't figure out if there is some error in the write-up or if we are not using exactly the same dishes or if we are doing something wrong, but the authors should see if they can address this in the text since I expect others will have the same problem.

The one other issue is that we weren't sure how to film multiple embryos. This is important particularly for analyzing experimental embryos or embryos from heterozygous crosses where only 1 in 4 are mutant. A careful explanation would help and perhaps this should be shown in the video.

Introduction
Line 58. Sentence is missing commas: "posterior body development at the level of molecules, single cells, and inter-tissue behaviors, as well as at the…"
- This has been corrected as highlighted in the text

Section 2

2.5 What size dish should be used?

- We have now added the dimensions of the dish to the main text, and the catalog number to the equipment list

2.6 How does this work if more than on embryo is in the dish at once? Not clear if they should be added in the same drop of agarose, or to put multiple different circles of agarose on the same dish. Additionally, perhaps a reminder that maintaining the lateral plane is important to the quality of the time lapse image. It is mentioned in the discussion but not previously.

- We have added a notes section where this has been described in more detail. It will also be demonstrated in the movie.

Section 3

3.3 Unclear about the description for cut 1- why does the cut begin posterior to the forming heart field- not clear from figure.

Also, would be help to give overview statement before the more detailed procedure- along the terms of "this cut is to remove the agarose from the posterior half of the embryo”

- A note has now been added at the beginning of section 3 that better describes the reasons for performing this procedure.

3.9 Note- Again, how would plating multiple embryos work?

- This note has now been fully described previously, so has been removed from this section.

Section 4

Note- This is a nice overview of the procedure- it would be nice to have a similar explanation at the beginning of Section 3

- This note has now been added

Figure 1- what diameter of glass ring on the bottom of the dish? It is not clear from the schematic. This was provided in the Comments section, but it would be informative to include earlier. Further, should the circle of agarose be contained to the 10 mm microwell? It seems like 1 mL is too large a volume to fit and the agarose flows over the sides of the microwell.

- We apologise for not having explained this step better. The embryo is taken up into the pasteur pipette with 1ml of mounting medium, but only around 100ul is expelled into the microwell. These details are now explained in the text.

Figure 2. B) final sentence has a typo- "Small coloured lines show tracks nuclei to floow individual cell movements" should be “follow"

- This has been corrected as highlighted in the text

Major Concerns:

N/A

Minor Concerns:

N/A

Additional Comments to Authors:

N/A

Reviewer #3:

Manuscript Summary:

The methods manuscript by Steventon and Hirsinger is concisely and clearly written. My major comment is whether the title could be more general, " A Versatile Mounting Method for Long Term Imaging of Zebrafish Development" and to perhaps discuss how this method could be used to image brain morphogenesis, for example. I realize that the authors have more experience imaging posterior body development, and they do mention that the technique could be used for imaging other processes. However, by altering the title and modifying the text, the article would likely gather more attention and be of use to a broader audience.

- We thank the reviewer for this suggestion and have altered the title, abstract and introductory text to open up the technique for researchers who are interested in the long-term imaginf of other aspects of zebrafish development.

I have one additional specific comment:
Sections 1.3-1.4. Is it necessary to use this specific capillary and pulling conditions? It seems that one simply needs a pulled needle in which the flexible tip is broken off. Perhaps a more general description (or image) of the desired tip shape would be more useful if it matters whether there is a long gradual taper or a short taper.
- In response to this comment and that of reviewer 1, we have added in the notes a statement that makes it clear that filament capillaries are not a specific requirement. In addition, the particular taper of the needle will be made evident in the accompanying instruction movie.

Major Concerns:
N/A

Minor Concerns:
N/A

Additional Comments to Authors:
N/A