Dear Editor,

We made the editorial changes you requested. We also thank the reviewers for their work.

Below are our responses in *green italics*:

**Reviewer 1**

We see the value in a labeled diagram and made changes accordingly.

* 64-65: ?.should read something like "for both the live and dead worms?.."
  + *inserted article ‘the’ as suggested and adjusted comma.*
* 117: ?.should read something like "this case study shows that the thrust?"
  + *change made*
* 118: first two commas should be taken out.
  + *change made*
* 121 - 126 Perhaps make two sentences here?
  + *Suggested change made*
* 160: Reference is made to video 1 in 2.3.1. I didn't understand at first why one needs a video here, but later (2.5) it became clear that it illustrates how the 5 cm line disappears when the light is turned off. Perhaps some additional explanatory note is in order.
  + *The video note is as directed by the editors of JoVE. This will stay as unless the editor directs other wise.*
* 179: Is Logger Pro the video analysis program?
  + *Yes, it is listed in the ‘supplies list’ as directed by JoVE we are not allowed to mention commercial products in the article.*
* 198: no comma after "file"
  + *We disagree with the reviewer here. A comma is placed since we are referring directly to the video file.*
* 203: This section (5.1.3) can only be understood by someone familiar with the operation of   
  the software.
  + *We agree with the reviewer. The editor wanted specific instructions on how to complete this task on this software without us mentioning the specific commercial product. We are not sure how to resolve this without acting against the reviewer’s request; however, the software is specified in the supplies list.*
* 225: replace "each," with "both".
  + *Change made*
* 273 - 276 The sentence that follows needs to be rewritten:  
    
  The worm starts with a significant upward thrust, slows down to start turning around at 68 s; however, the continuous decrease of upward acceleration (Fig. 8) [Place Figure 8 here] until the net acceleration equals zero around 68.5 s.
  + *Yes, we see the issue with this sentence and changed it.*
* 313: Presumably they mean that there is no drag, and that the thrust is pointing up.
  + *Yes, we made a change but are not sure what the reviewer wanted changed here.*
* 322: "eventually has" rather than "has eventually"
  + *change made*
* 325: This caption needs clearer punctuation.
  + *change made*
* 339: don't need comma after "forces".
* *We disagree with the reviewer here. A comma is placed since we are referring directly to the forces.*
* **Reviewer 2**

This manuscript describes a method for tracking the movements of C. elegans in a liquid environment using laser illumination and image analysis. The authors measure the descent rate of live and dead worms and estimate the forces exerted during swimming.  
  
I have two concerns about this manuscript: First, there are major mathematical and conceptual errors in the analysis. For example, the estimate of mass is incorrect by about 3 orders of magnitude,

*Our calculation could be rounded up one order of magnitude depending on the assumptions made. The reviewer does not explain or give a reference how he/she arrives at the indicated mass error. We arrived at the mass estimate by assuming that the worm is cylindrical, roughly 1mm long and 100 microns in diameter. This assumption leads to a worm volume of about 1 x 10-8 m3. Assuming that the worm consists mainly of water, the density can be assumed to be 1 kg/decimeter3. This gives a mass of 10-8 kg, which is 10-5 g or 0.01 mg or 10 micrograms, which is cannot even be considered an error of one order of magnitude since our initial estimate was 3 micrograms. We clearly state that this is an estimate and not the focus of this work. We do not think that this is worth expanding past the allowable length of the manuscript; however, we are clarifying in the text that we are assuming that the density of the worm is roughly that of water.*

and the discussion of force balance neglects the buoyancy of the water, resulting in another error of about 2 orders of magnitude. These are, however, errors which could be corrected.

*We recognize that buoyancy should be addressed explicitly. The reviewer does not offer an explicit reasoning for his/her conclusions. We have made the adjustments and necessary corrections in the manuscript as we see it.*

A more serious problem is that method is poorly motivated and does not seem very useful. The emphasis on single wavelength imaging is perplexing, as multiple wavelengths do the job equally well, if not better. The discussion of the limitations of "conventional microscopes" is highly misleading (as detailed below). In fact, everything described in the manuscript could be done much more easily and inexpensively using a simple dark-field illuminated cuvette and a webcam-quality video camera. It seems unlikely to me that any scientists will find this technique helpful for their research.   
  
Detailed comments:  
  
"This study demonstrates an inexpensive and straightforward technique that allows the measurement of physical properties such as position, velocity, acceleration and forces involved in the locomotory behavior of nematodes suspended in a column of water in response to single wavelengths of light. "  
  
This sentence seems to imply that nematode locomotion occurs in response to laser illumination. This doesn't make sense and I don't think it was intended.

*The reviewer is mistaken in his/her assumptions. We did mean to suggest that this method is a cheap and easily accessible method to conduct measurements in* ***various types of orientations*** *in response to* ***highly selective wavelengths****. We challenge the reviewer to provide the make and model of even one commercially available microscope under $100,000.- that is capable of using any commercially available laser wavelength.*  
  
"It is currently unknown how C. elegans respond to different types of environments stimulating various types of locomotory patterns"

This overly broad statement neglects a large body of literature connecting mechanical, thermal, olfactory, and other sensory stimuli with behavioral responses in C. elegans. The authors should reference some of the many relevant papers on worm behavioral tracking.

*We acknowledge that our statement seems to overstate things by being too general. We have modified our statement to be more specific to the goals of our project.*

*“While much is known about the neural circuitry of sensorimotor function and general locomotory patterns in C. elegans, less is known about the responses to multiple, concurrent stimuli or more complex environmental conditions than can be modeled under a microscope. A few studies have revealed more complex locomotory patterns that are highly plastic (Pierce-Shimomura et al, 2008; Berri et al, 2009; Vidal-Gadea et al, 2012). Our methodological approach will enable studies of nematodes in solution in real time where we can readily provide multiple environmental conditions simultaneously.”*

"Single Wavelength Shadow Imaging (SWSI) is presented in this paper for the first time to address the shortcomings of traditional microscopes."  
  
It is not demonstrated how single wavelengths are helpful here. If anything the long coherence length of the laser source seems to create unnecessary fringes and noise in the image.

*The reviewer is completely ignoring the remainder of the paragraph and the references to which he/she is referring. There are behavioral studies which have studied locomotory response with limited wavelengths using conventional light microscopy.*  
  
"Traditional microscopes are limited to observe species in a horizontal focal plane a few microns in depth"  
  
Depth of field is determined by the numerical aperture of the imaging system, and can be as large as several centimeters with appropriate optics. For a relatively large field of view considered here, it is not necessary to use microscope optics. A conventional camera lens does quite well and is capable of a large depth of field.

*The reviewer is partially correct here neglecting that there is a trade off for depth of field. Also, conventional cameras don’t allow for single wavelength studies.*

"A reasonable estimated mass is 3 <mu>g." Typical dimensions of a young adult worm are 1 mm length and 60 micron diameter. Assuming a density close to water, a worm has a mass of about 3 ng, three orders of magnitude smaller than stated.

*Following the calculations presented earlier in this rebuttal, the reviewer made a calculation error even assuming a 60 micron width.*  
  
The buoyant force due to water displacement is neglected in the force balance. If worms had a density equal to that of water, there would be no net force due to gravity. For estimates of worm density the authors may see a recent paper from George Whitesides' lab.

*We are grateful for pointing out this error and made appropriate corrections in this manuscript.*  
  
"The first investigation shows no distinguishable difference in the descending rates of the C. elegans during SWSI using 633 nm"  
  
The authors need to perform a statistical test to justify this conclusion.

*We are presenting a method here and not an extensive biological study. The data presented are not without value and is representative of the method as required by JoVE.*  
  
Figure 1: Where is the camera positioned?

*We have included explicit instructions in procedure item 2.1.*  
  
Materials: The camera is described in the text as Nikon Coolpix and in the appendix as Casio Exilim. If two different cameras were used, please state this.

*This issue was corrected during an earlier editorial review.*