

Response to Editorial Comments

Thanks for the comments on our manuscript. We have provided point-by-point responses below.

Note that the manuscript has been reformatted somewhat and the protocol sections have been renumbered (with an introductory note on safety); all the cross-references should be correct.

We have corrected cross-references as needed.

1. It is difficult to pick out the instructions from the critical points for your longer steps. Please split these up into substeps in the imperative and ‘Notes’; e.g., for 1.1.2.1:

“1.1.2.1. Attach a lens to the PIV camera, turn-on the PIV camera in free/continuous mode, and coarsely focus the PIV camera.

Note: The f-stop of the PIV camera may need to be adjusted to allow sufficient light to be received by the imaging sensor; this f-stop setting may differ when using the room’s white light versus the laser-based illumination. Selection of the lens and the distance between the PIV camera and the light sheet determines the physical size of the PIV camera’s image.

1.1.2.2. Adjust the lens and distance until the size of the image is sufficient to observe the region of interest. Ideally, the size of the image should be smaller than (or similar to) the size of the light sheet setup in 1.1.1.”

We have separated the ‘Notes’ from the instructions for the longer steps.

2. The software instructions you have here seem sufficient (although, note that the shooting script will be generated entirely from what is highlighted in the manuscript), but listing specific software to use would be highly helpful to the reader, especially given that all the reviewers brought it up. Can you at least add a “Note” (with citations) to the beginning of section 2 saying what software you used and some (ideally non-commercial) alternatives?

Thanks. We have added a brief note at the top of section 2, listing some options (with focus on freeware). We have also added the software platforms we used.

“Note: There are numerous software packages available to perform both the PIV and PT image analysis – both commercial and freeware. For PIV analysis, freeware codes are OpenPIV (<http://www.openpiv.net/>) and MatPIV (<http://folk.uio.no/jks/matpiv/index2.html>). Commercial companies also sell PIV analysis software such as TSI, Inc. For PT analysis, numerous particle tracking codes exist in both 3D and 2D such as Particle Tracker (<https://omictools.com/particle-tracker-tool>); a full listing of various software platforms can be found here:

<https://omictools.com/particle-tracking-category> or <http://tacaswell.github.io/tracking/html/>. Most analysis packages, e.g., MATLAB, have built in tools that make it relatively easy to implement your own tracking code. For the results presented in this study, OpenPIV, TSI Insight, and MATLAB custom-written tracking codes were used.”