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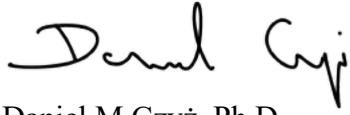
August 23, 2021

JoVE

Dear Dr. Krishnan –

Please find attached a revised version of manuscript # JoVE62997 entitled "Automating Aggregate Quantification in *Caenorhabditis elegans*." We would like to thank you for the opportunity and allowed time to revise our manuscript. We include a point-by-point response to each reviewer's comments and have updated the manuscript accordingly with the recommendations offered by you and the reviewers.

The revised version of the manuscript includes several of the experimental recommendations suggested by the reviewers. We have included new data that demonstrate technique optimization and reproducibility. In particular, we showed the effect of sample freezing on aggregate enumeration (new Figure 3). We have developed a Graphical User Interface (new Figure 8) software (for Mac and Windows OS) that allows us to automate the processing of large excel datasets generated using CellProfiler. While we did not feel it was appropriate to expand our approach using additional models because of the extensive optimization that would expand the manuscript, we did demonstrate its feasibility to detect muscle-specific polyQ (new Supplemental Figure 1). Furthermore, we demonstrated reproducibility between individual experimenters by having three separate individuals collect data from the same samples (new Supplemental Figure 2) . We also acquired images and analyzed data from the same worms positioned in 15 different orientations (new Supplemental Figure 3). Additional controls, such as the effect of FUDR on aggregation, were also added (new Supplemental Figure 5).

As a result of these additional experiments, we have added two additional authors to the manuscript: Carol Navya Pagolu and Samantha M. Enslow, who contributed to revision experiments. All authors agreed to and acknowledged the addition.

As stated in our initial submission, we believe our study will be of interest to those who employ *C. elegans* as a model to study the effect of bacteria on proteostasis and those investigating other factors, conditions, and small molecules. Please see the attached document titled "Reviwe_Response," which addresses each issue raised by the reviewers. Thank you very much for handling the review process and for providing guidance on how to make our study suitable for publication in JoVE. We very much appreciate the time and attention you have devoted to this submission.

Yours sincerely,

Daniel M Czyż, Ph.D.
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