



October 9, 2018

Dear Editor,

Please find enclosed a revised manuscript entitled “Monitoring neuronal survival via single-cell longitudinal fluorescence microscopy” for consideration of publication in *The Journal of Visualized Experimentation (JoVE)*. This resubmission (previously titled “Monitoring neuronal survival via longitudinal fluorescence microscopy”) is in response to an invitation from Ronald Myers, Science Editor at *JoVE*. In this manuscript, we provide detailed information on automated or longitudinal fluorescence microscopy, complete with a step-by-step protocol for conducting experiments involving automated microscopy. We also provide sample worksheets and scripts for data processing and analysis to facilitate the adoption of this method where needed.

In response to comments and suggestions from the editor and reviewers, we have made several revisions to the manuscript:

- The title has been changed to “Monitoring neuronal survival via single-cell longitudinal fluorescence microscopy” to reflect the unique ability of this technique to monitor individual cells over time.
- We expanded the introduction and revised Figure 1 to better illustrate the concept of longitudinal fluorescence microscopy.
- We added new references describing conventional methods for determining cell death and criteria for judging time of death.
- We discuss alternative possibilities to cell death, including the movement of cells and the delayed appearance of cells over the experimental time course.
- All auxiliary verbs have been removed and replaced by imperatives where possible.
- All references to company names or trademarks have been removed from the text.
- An appropriate ethics statement is now included within the text.
- The protocol now includes references to in-depth protocols where necessary.
- The protocol is now more streamlined, with additional information provided within notes or moved to the Representative Results and Discussion sections.
- The Discussion section now includes a list of critical steps that can be modified for individual applications.
- The table of essential supplies has been modified to include company names where needed.

We are confident that these revisions have strengthened the manuscript and clarified the protocol, ultimately making it easier for readers to follow and recapitulate. No portion of this manuscript or the attached documents have appeared in print or been submitted for publication elsewhere. Thank you for your consideration of this manuscript.

Please do not hesitate to contact me with questions or concerns.

Sincerely,

Sami Barmada, MD, PhD  
Assistant Professor of Neurology and  
Angela Dobson and Lyndon Welch Research Professor of Neurology