**In this revision letter, we highlighted our revision in red below each editorial and reviewer’s comment.**

**Editorial comments**

The manuscript requires thorough copyediting for grammar and formatting, ideally by a native English speaker.

The revised manuscript has been copyedited by two native English speakers.

-Throughout the manuscript, SI units should be used (e.g. mL rather than ml) and units should not be crashed. At present the units are inconsistently formatted.

Changed: all ml to mL

-The micro- prefix should be indicated with the mu symbol (μ) rather than a letter u.

Changed: all um to µm, uL to µL

-Ranges of values (e.g. 0.13-0.17 mm) should be indicated with a hyphen rather than a tilde (~).

Changed: 0.13~0.17mm to 0.13-0.17mm

-Step 2.1: "Place a clean weigh boat on a balance and tare the balance, pour 20g PDMS base into the weigh boat." - This should either be split into two sentences, or needs an "and."

As suggested, this has been split into two sentences.

-Step 2.3: "Using a disposable pipette to stir the PDMS base and the curing agent."

Changed to: Stir the PDMS base and the curing agent with a disposable pipette

-Step 2.4: "Pour the mixture slowly into the Petri dish, the mixture must cover the silicon wafer mode completely."

Changed to: Pour the mixture slowly into the Petri dish. The mixture must cover the silicon wafer mode completely.

-Step 2.7: "Peel the layer of polymerized PDMS off the silicon wafer mold gently, avoid[ing] any damage to the construction of the wafer mold. "

Changed to: avoiding any damage to the construction of the wafer mold

-Step 2.8: "Place the PDMS layer on the bench with pattern side facing up, carefully cut out the individual chip with a single edge industrial razor blade, retain adequate margin from the edge of a single chip to avoid construction damage."

Changed to: Place the PDMS layer on the bench with the pattern side facing up. Carefully cut out the individual chip with a single edge industrial razor blade, retaining adequate margin from the edge of a single chip to avoid construction damage.

-Step 2.10: "Check every punched hole by inserting the punch pen needle again into the hole, if the needle can come out from the other side, indicates there is no blockage."

Changed to: Check every punched hole by inserting the punch pen needle again into the hole. Make sure the needle can come out from the other side, indicating there’s no blockage

-Step 2.12: "The glass can additionally be washed with sterilized water and then dry with dust remover." (Should probably be "dried")

Changed to: The glass can additionally be washed with autoclaved water and then dried with dust remover.

-Step 2.13: "Transfer the cover glass and PDMS in a plastic plate, " Should this be "to" a plate?

Changed to: Transfer the cover glass and PDMS to a plastic plate

-Line 165: "Deficient microfluidic can cause medium leakage into the microscope..."

Changed to: Deficient microfluidic may cause liquid leakage onto the microscope during the experiment. Therefore, it is important to check the bonding between the PDMS and the cover glass by slightly lifting the PDMS from the edges with a tweezer. Gently lifting the PDMS should not separate it from the cover glass, indicating a successful bonding.

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-Step 3.1.2: "Full fill 5 ml syringe with autoclaved water..."

Changed to: Fill a 5 mL syringe with autoclaved water to wash the tubes. Wash each tube by plugging the tube on the syringe and flushing the tube with water.

-Step 3.2: "Make multiple chips at one time, use the rests as backup chips especially if you are making the device for the first time."

Changed to: Make multiple chips at one time to ensure there are backup chips - especially if you are making the device for the first time.  
-Lines 218-219: One can select one of the many freely available image processing tools to analyze the images, for example, the ImageJ and the plugins."

-Line 221 should be rewritten to avoid using second person ("you").

Changed to: Since there are a number of different features that can be analyzed for each cell, the first step of the analysis is to annotate the cells and events, including the positions and boundaries of the cells and the timing of various events that are being tracked, for example, the budding events. These annotations will make it easier to return to the same set of cells and analyze different features in the future. A list of phenotypes can be then extracted from the image data using the recorded annotations of the cells with image analysis software such as ImageJ11 and the plugins.

-Figure 2 heading reads a bit awkward: "The dynamics of budding intervals as cell aged."

Changed to: The length of budding intervals changes as cell aged.

-Line 260, "Fluorescent" does not need to be capitalized here.

Changed to: fluorescent  
  
•Unnecessary branding must be removed:  
-Step 2.14 - Plasma Tech, Inc.

Changed: the branding has been removed

-Figure 3 legend - The MathWorks, Inc.

Changed: removed

•The first paragraph of the Results section might be better moved to the Discussion, although this is not required.

We did not change this.   
  
•If your figures and tables are original and not published previously, please ignore this comment. For figures and tables that have been published before, please include phrases such as “Re-print with permission from (reference#)” or “Modified from..” etc. And please send a copy of the re-print permission for JoVE’s record keeping purposes.

Our figures are original.

•JoVE reference format requires that the DOIs are included, when available, for all references listed in the article. This is helpful for readers to locate the included references and obtain more information. Please note that often DOIs are not listed with PubMed abstracts and as such, may not be properly included when citing directly from PubMed. In these cases, please manually include DOIs in reference information.

Changed: We double checked the three references with a DOI information and add the DOI for all of them manually.

•IMPORTANT: Please copy-edit the entire manuscript for any grammatical errors you may find. The text should be in American-English only. This editing should be performed by a native English speaker (or professional copyediting services) and is essential for clarity of the protocol and the manuscript. Please thoroughly review the language and grammar prior to resubmission. Your JoVE editor will not copy-edit your manuscript and any errors in your submitted revision may be present in the published version.                                                             
  
•NOTE: Please include a line-by-line response letter to the editorial and reviewer comments along with the resubmission.

**Reviewers' comments:**  
**Reviewer #1:**  
*Manuscript Summary:*  
N/A  
  
*Major Concerns:*  
1. The authors write: "In our experiments, our device frequently traps fresh mother cells, which budded from already trapped cells." How is this established? What fraction of the cells are fresh mothers? At the moment, it is just an assertion by the authors.

We have modified the manuscript in the discussion adding the estimated ratio of fresh mother cells observed from our experiments and a brief description on the method to obtain the numbers.

2. This paper would be considerably more useful if the authors provided the CAD files for the masks described in steps 1.1, 1.2, and 1.3. Otherwise, the likelihood that researchers will use the protocol is small.

We have provided the CAD file for the mask used in this protocol.

3. The description of the soft lithography process is both short and incomplete.

We have added more descriptions of the soft lithography process and cited references for the standard procedures.

4. The authors don't make any comments on the robustness of the cell traps. What's the frequency at which mother cells are retained/lost? This is an important parameter of interest for those who might consider implementing this protocol.

We have added the retention rate of trapped cells estimated from multiple experiments in the discussion. We also discussed the key parameters to ensure a robust cell retention.   
  
Minor Concerns:  
I recommend altering the title of the paper slightly to read "Using Microfluidic Devices To Measure Lifespan and Cellular Phenotypes In Single Budding Yeast Cells".

Changed to: "Using Microfluidic Devices To Measure Lifespan and Cellular Phenotypes In Single Budding Yeast Cells"

Additional Comments to Authors:  
N/A  
  
  
Reviewer #2:  
Manuscript Summary:  
This manuscript describes a microfluidic method to determine yeast replicative lifespan. Detailed steps are provided from wafer preparation to PDMS device fabrication, to microfluidic operation. This manuscript will provide necessary technical references for researchers who want to adopt the microfluidics-based method to measure yeast replicative lifespan.  
  
Major Concerns:  
The manuscript should provide a flow chart to overview the experimental process and a microfluidic design schematics. Microfluidic design should be described in detail and the design file should be provided.

We have added a new figure 1 for the microfluidic device design schematic and provided the CAD file for the mask used in this protocol. We feel that it is unnecessary to provide a flow chart since the experimental steps have been described in details in the protocol.

Minor Concerns:  
N/A  
  
Additional Comments to Authors:  
N/A