

**Manuscript No: JoVE60185**

**Manuscript title: Generation of heterogeneous drug stress gradients across cancer populations on the microfluidic Evolution Accelerator for real-time observation**

**First and corresponding author: Ke-Chih Lin**

Dr. Phillip Steindel, Review Editor  
Journal of Visualized Experiments

Dear Dr. Steindel,

We thank the reviewers for their thorough reading of our work and forward-looking suggestions. We have responded appropriately by adding additional information on the experimental methods and comments in the manuscript.

We have attached responses to each of the reviewers as well as the revised manuscript. Please don't hesitate to email me if I can be of further assistance.

Sincerely,  
Ke-Chih Lin

**Editorial comments:**

General:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.
2. Please ensure that the manuscript is formatted according to JoVE guidelines—letter (8.5" x 11") page size, 1-inch margins, 12 pt Calibri font throughout, all text aligned to the left margin, single spacing within paragraphs, and spaces between all paragraphs and protocol steps/substeps.
3. Please reduce the length of the summary to be between 10 and 50 words.
4. For in-text formatting, corresponding reference numbers should appear as numbered superscripts (without brackets) after the appropriate statements.
5. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please limit the use of commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents. For example: L-Edit, Heidelberg DWL66, AZ 300 MIF, Microposit Remover 1165, Cr-7 Chromium Etchant, Lumox™, etc.

(1) We have proofread the manuscript again.

(2) The manuscript is now formatted according to JoVE guidelines.

(3) The summary is now shortened (49 words).

“We present a microfluidic cancer-on-chip model, the “Evolution Accelerator” technology, which provides a controllable platform for long-term real-time quantitative studies of cancer dynamics within well-defined environmental conditions at the single-cell level. This technology is expected to

work as an in vitro model for fundamental research or pre-clinical drug development.”

(4) The format of reference numbers is now corrected.

(5)

The commercial terms have been removed and replaced with generic terms as follows:

L-Edit: removed

Heidelberg DWL66 laserwriter: laserwriter

Microposit Remover 1165: photoresist stripper

Suss MA6 mask aligner: mask aligner

Lumox™: gas-permeable culture dishes/membranes

Samco RIE800iPB DRIE: Silicon Deep Reactive Ion Etching (DRIE) systems

TePla M4L plasma etcher: plasma etcher

Polymethylsiloxane (PDMS) Sylgard 184: Polymethylsiloxane (PDMS) kit

10 mL BD Luer-Lok™ tip syringes: 10 mL syringes

Luer-Lok™ 23 G dispensing needle: 23 G dispensing needle

Microseal B Adhesive Sealer: adhesive sealer

Ibidi system: on-stage incubator

And the materials used in the manuscript have been referenced to the best of our knowledge.

Protocol:

1. For each protocol step, please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. If revisions cause a step to have more than 2-3 actions and 4 sentences per step, please split into separate steps or substeps.

The protocol has been revised as suggested.

Specific Protocol steps:

1. 1.1: Can you include this pattern as supplementary material?

2. 3.1.1, 3.3.1: Please include more information about the preparation of the plate holder and gas supply system.

(1) The EA pattern template files (“EA\_large-gap.gds”, “EA\_small-gap.gds”) and the 3D CAD file (“3-well plate.FCStd”) are now included in the supplementary materials and on GitHub.

(2) Protocol 3.1 is reorganized and supplemented. In protocol 3.3.1, our previous work is now referenced to provide more details.

Figures:

1. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account.

2. Please remove the embedded table from the manuscript.

3. Please remove the embedded figure(s) from the manuscript.

4. Please consistently use either ‘h’ or ‘hours’ in the figures (not ‘hr’ or ‘hour’).

5. Figures 6c: Please define the error bars in the legend.

(1) The copyright permission of IOP Publishing obtained via Copyright Clearance Center (CCC) is uploaded as the submission material (“copyright permission.docx”).

(2) (3) The embedded table and figures are removed from the manuscript.

(4) Only 'h' and 'hours' are used in the figures now.

(5) The error bars in Fig. 6 C is now defined in the legend:

“The error bars represent the standard deviation of 5 experiment samples.”

#### Discussion:

1. Discussion: As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3–6 paragraphs with citations:

- a) Critical steps within the protocol
- b) Any modifications and troubleshooting of the technique
- c) Any limitations of the technique
- d) The significance with respect to existing methods

(a) Critical steps are now covered in the 3<sup>rd</sup> paragraph.

(b) Troubleshooting and modifications are now covered in the 3<sup>rd</sup> and part of the 5<sup>th</sup> paragraphs.

(c) Limitations are now covered in the 4<sup>th</sup> paragraph.

(d) The significance with respect to existing methods is covered in the 1<sup>st</sup> and the 2<sup>nd</sup> paragraphs.

#### References:

1. Please ensure that the references appear as the following: [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source. Volume (Issue), FirstPage – LastPage (YEAR).] For more than 6 authors, list only the first author then et al.

(1) The format of the references is corrected.

#### Table of Materials:

1. Please ensure the Table of Materials has information on all materials and equipment used, especially those mentioned in the Protocol.

The materials used in the manuscript have been referenced to the best of our knowledge.