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Jaydev Upponi, PhD
Editor
Journal of Visual Experimentation
1 Alewife Center, Suite 200
Cambridge, MA 02140

April 30, 2021

Dear Dr. Upponi:

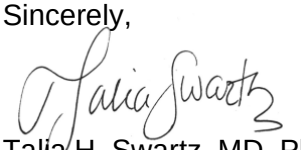
Thank you and the reviewers for your thoughtful suggestions to revise our manuscript entitled "A multiparameter optofluidic platform for single-cell characterization of early and late events in HIV-1 infection" for consideration as a Journal of Visual Experimentation Research Article.

We have made the requested edits in this resubmission and indicated the rebuttal to follow.

Many thanks for your input and looking forward to being in touch.

Thank you for your consideration and we look forward to your feedback.

Sincerely,



Talia H. Swartz, MD, PhD
Assistant Professor of Medicine
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Editorial comments:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

This has been done.

2. Please revise the following lines to avoid previously published work: 133-136, 139-143.

We have not included any previously published work.

3. Please revise the text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

This change has been made.

4. Please define all abbreviations before use (MFI, etc.)

All abbreviations have been defined and a new section on abbreviations has been added.

5. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials.
For example: Beacon, Berkeley Lights, Beacon OptoSelect, PowerLoad concentrate, Pluronics, Prism, etc.

This change has been made.

6. Please rephrase the Summary to clearly describe the protocol and its applications in complete sentences between 10-50 words: “Here, we present a protocol to …”

The new summary reads:
“Here, the authors present a protocol in which single cells are monitored for acute events and productive HIV-1 infection on a nanofluidic device. Imaging data define virus-host receptor interactions and signaling pathway dynamics. This is the first method for nanofluidic high-throughput longitudinal single-cell culture and imaging to study signaling kinetics and molecular interactions.”

7. Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary. Please refrain from using bullets or dashes.

This change has been made.

8. Please add more details to your protocol steps. 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.

This change has been made.

9. Please replace “uL” and “uM” with “µL” and “µM” throughout the protocol, respectively.

This change has been made.

10. Line 190: How is penning performed?

“Penning occurs using optimized optoelectronic positioning (OEP).” This has been added to line 324-25.

11. Line 200: Please specify how is HIV-1 infused into the microchip. What is used for infusing?

“Infuse HIV-1 into the microchip at a concentration of 13 ng HIV-1 NL-CI per 2 x 10^6 cells by slowly pipetting the suspension into the chip through the export needle.” This has been added to line 334-35.

12. Please highlight up to 3 pages of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

This has been done.

13. 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. Title case and italicize journal titles and book titles. Do not use any abbreviations.

This change has been made.

14. For in-text formatting, corresponding reference numbers should appear as numbered superscripts after the appropriate statement(s).

This change has been made.

15. Figure 1: Please Maintain a single space between the numeral and (abbreviated) unit, except in cases of %, x, and ° (i.e., the degree sign; excluding temperature). Examples: 5 mL, t8.5 min, t4 days, etc. For time units, use abbreviated forms for durations of less than one day when the unit is preceded by a numeral. Do not abbreviate day, week, month, and year. Examples: 5 h, 10 min, 100 s, 8 days, 10 weeks

This change has been made.

16. Figure 2/3: Please define the units in the Y-axis.

This change has been made.

17. Figure 4: Please define the units in the X and Y-axis.

This change has been made.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

This is a very interesting paper describing a method using optofluidic fluorescence microscopy for automated single-cell detection. This technique can be broadly adapted to study cellular signaling kinetics and dynamic molecular interactions. I believe this paper is worth filming a video for broad audience.

Thanks to the reviewer for this kind assessment.

Reviewer #2:

Manuscript Summary:

The authors present a new application of the Beacon Optofluidic System which involve simultaneously monitoring P2XR activity and HIV infection in individual cells. The P2XR activity is monitored using calcium sensitive dye whereas the HIV infection is monitored using a mCherry fluorescent reporter virus.

Major Concerns:

The Beacon opto-fluidic platform used here is interesting and may be useful for multiple different applications, in addition to HIV related studies. However, there are some questions that need to be addressed in this manuscript. The authors claim that "single-cell imaging with standard fluorescence microscopy is low-throughput". But it is unclear how exactly does the opto-fluidic platform increase the throughput of analysis. The authors should provide quantitative data to support this claim. The throughput will depend on the NA of the objective used, irrespective of the platform. Does the Beacon system have a wide scanning and stitching ability? A detailed description of the OptoSelect chip would be helpful, e.g. (1) what is the efficiency of positioning cells in individual wells?, (2) how many cells can be imaged at a time? etc. Why are there so few cells in image 1? It would be good to show the actual high throughout nature by imaging as many cells as possible. What is the sensitivity and limit of detection of the system for detection of Ca? Please describe as to how this technique is scalable, as claimed in the abstract? Another question is regarding the software. Is it automated (as mentioned in the abstract) or do the authors manually do the analysis using the ImageJ macros?

Thanks to the reviewer for these helpful questions and suggestions.

More detail has been added:

> The optofluidic system has wide scanning and stitching ability. The optical sorting on the chip has high efficiency that is variable depending on cell type, but automatic sorting efficiency is between 80-90% and manual sorting can increase that efficiency to 100%. Each chip can accommodate 3500 cells and four chips can be run simultaneously, with a parallel workflow, resulting in high scalability. While the imaging workflow is reported by manual image analysis, more recent updates in the system allow for automatization of this. It is important to note that the data shown here are representative within the limitations of image threshold analysis, and optimization and automatization of image analysis are likely to result in more sensitive measures of both calcium influx and mCherry signal as indicative of HIV-1 productive infection.

Reviewer #3:
Minor Concerns:
In this study, the authors reported their latest method article titled 'A multiparameter optofluidic platform for single-cell characterization of early and late events in HIV-1 infection' in the Journal of Visualized Experiments. They described a method that applies principles of live-cell fluorescence microscopy to an automated single-cell platform that cultures and images cells over user-customized time courses, allowing for high throughput analysis of dynamic cellular processes. This manuscript is correctly set up, and well-written. The manuscript can be accepted with minor revisions.
1. The abstract part can be shortened.
2. The introduction part's paragraphs should be rephased to connect each other.

Thanks to the reviewer for these helpful questions and suggestions. The abstract has been shortened and the introduction paragraphs have been rephrased to better connect to each other.

Reviewer #4:
Manuscript Summary:
This manuscript is describing a multiparameter optofluidic platform for single-cell characterization of early and late events in HIV-1 infection. The results are interesting and convincing. The developed platform provides a lot of visual information that can be further analyzed by researchers. There are many additional applications of this technology including understanding of cellular signaling kinetics and dynamic molecular interactions. Overall, this is an excellent piece of work.

Thanks to the reviewer for this kind assessment.