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16 Oct 2020

Editors
Journal of Visualized Experiments

Dear Editors:

We have addressed the comments raised by the reviewers point by point. We have marked the changes in RED color in the revised manuscript.

Comment to Editor:
We have made extensive correction in this revision.

Editorial comments:

Changes to be made by the Author(s):
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

> We have made sure the paper is thoroughly proofread.

2. Please format the manuscript as: paragraph Indentation: 0 for both left and right and special: none, Line spacings: single. Please include a single line space between each step, substep and note in the protocol section. Please use Calibri 12 points

> We have made sure this is the case.

3. Please provide at least 6 keywords or phrases.

> We have provided at least 6 keywords.
4. Please ensure that the long Abstract is within 150-300-word limit and clearly states the goal of the protocol.

> We have made sure this is the case.
5. Please define all abbreviations during the first-time use. e.g. PCB, EWOD, MOSFET, CMOS, FET, etc.

> We have define the abbreviations during the first time use.

6. Is OpenDrop platform commercial? If yes, please use generic term as we cannot have commercial term in the manuscript.

> OpenDrop is an academic term from academic papers. They are made available through the Gaudi'group so it is not really commercial in the usual sensce. We wish to reserve the term as it is most easily understood and identified within the community. The same is true for DropBot.

7. JoVE cannot publish manuscripts containing commercial language. 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 and Reagents.
For example: BSS131, Infineon SIPMOS, BSP89 H6327 Infineon, etc.

> We have completely removed these commercial terms.

8. Please ensure the Introduction include all of the following with citation:
a) A clear statement of the overall goal of this method
b) The rationale behind the development and/or use of this technique
c) The advantages over alternative techniques with applicable references to previous studies
d) A description of the context of the technique in the wider body of literature
e) Information to help readers to determine whether the method is appropriate for their application

> We have completely revised the introduction to provide comparison between different methods and all the aforementioned requirement.

9. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note."
10. The Protocol should contain only action items that direct the reader to do something.
11. Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed?
12. There is a 10-page limit for the Protocol, but there is a 3-page limit for filmable content. Please highlight 3 pages or less 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.

> We have completely revised protocol to make sure this is fulfilled.

13. Please expand the discussion on figure 2 and figure 3 in the representative result section.

> We have expanded the discussion on Figure 2 and Figure 3.

14. 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. The Figure must be cited appropriately in the Figure Legend, i.e. "This figure has been modified from [citation]."

> This is not applicable.

15. As we are a methods journal, please ensure that the Discussion 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
e) Any future applications of the technique

> We have completely revised Discussion to make sure these requirements are fulfilled.

16. Please include all the Figure Legends together at the end of the Representative Results in the manuscript text.

> We have made sure figure legend are included.

17. Please upload each Figure individually to your Editorial Manager account. Please combine all panels of one figure into a single image file. Please remove the figure numbers from the figure.

> We have made sure figure legend are included.

18. Please sort the materials table in alphabetical order.

> We have made sure the material table is sorted to follow the alphabetical order.

19. A minimum of 10 references should be cited in the manuscript.

> We have cited more than 10 references.

Reviewer #1:

Manuscript Summary:

The authors describe a protocol to build a platform for electrowetting driven microfluidics. Electrowetting has become a widely used method to mobilize small amounts of liquid over surfaces in controlled environments. The authors emphasize the low cost of the materials and propose that the described set up may be accessible to instructors and students in an educational setting. Advances in microfluidics in the last two decades have not shown the initially expected impact in some fields (e.g. the biological sciences), and it is thought that lack of accessibility and cost in both the classroom and the non-specialist research lab is a significant barrier to wider adoption. Platforms that lower cost and expertise needed to adopt these technologies are valuable additions to the scientific toolbox. The authors provide building instructions and code ready to use by potential users, as well as examples of how the platform may be used. The manuscript is generally well written and has a good flow, but modifications might be needed to facilitate its implementation in the classroom by a wide, non-expert audience.

Major Concerns:

1. More detailed explanations and use of Notes will help those with limited expertise in electronics. The protocol seems to gloss over some of the required steps. In other words, the authors assume a certain degree of expertise in electronics by the user. For example, the manuscript seems to assume experience with Arduino microcontrollers. What computing platform and software are required to run the custom script? Some students and instructors may not know how to use a multimeter. Although it would be unreasonable to expect instructions on how to solder components together or how to use the multimeter, perhaps the authors can establish from the beginning what is expected from the user in terms of familiarity with electronics. If the goal is to facilitate the use of this technology in the classroom, one would expect that a chemistry or biology instructor, at least at the college level, may be able to build this platform following a more detailed protocol described in the manuscript, one that does not skip steps by assuming specialized knowledge.

>This is a very good point. The video format will solve questions of how to use multimeter automatically. We will demonstrate how to load the custom script as much as we can as long as it is compatible with the video format.

We have also solicited undergraduate students to perform a test of assembly the kit and the success rate is included in this revision. The success rate (~60%) clearly shows that in the worse scenario,

undergraduate students can assemble the kit themselves without any prior training in soldering or electronics.

2. The list of materials is incomplete (e.g. battery array, wire, acrylic box, etc.) and lacks prices/cost for many items. The last point is relevant since the authors make a claim that this platform is approximately 10x cheaper than a comparable one. There is also a need for a list of the equipment/tools that will be required to assemble the device.

> We update the list of materials to make sure components are included with cost.

3. The introduction can benefit from a brief description (for the non-expert) of electrowetting in microfluidics and provide examples that illustrate how it relates to concepts taught in physics, chemistry or engineering (e.g. contact angle, electrocapillarity).

> We have added a description of electrowetting physics in introduction. We include a short paragraph to explain the physics of droplet actuation in the discussion session.

Why would an educator be interested in using the kit?

If the goal is to find this set up in the classroom, to what kind of lessons can instructors add this tool?.

>Also, we add recommendation on how this kit can be used in the concluding paragraph in the Discussion.

"More specifically, the kit allows students to learn optics, electronics, and fluidics so this specific is suitable for any lab course in electrical engineering and mechanical engineering. Also, the specific chemiluminescence can be employed in a chemistry or chemical engineering experimental course at senior level."

A simple paragraph explaining electrowetting and how an instructor can use the kit to teach may be enough to make the case for this being an "educational kit" and not just another way to build an electrowetting platform.

> We include a short paragraph to explain the physics of droplet actuation in the discussion session.

"Here we briefly discuss the physics involved for droplet actuation. …….In our experiment, we use dc actuation and hence the operation is below this critical frequency and hence the three-phased contact line is electrostatically pulled toward the actuated electrode."

Minor Concerns:
Is oil applied in both step 1.4 and step 1.5? Perhaps add a note explaining the purpose.

>We have added explanation. In step 1.4, oil is used to avoid air gap. In step 1.5, the oil serves as hydrophobic layer.

Is that a breadboard in Supplementary Figure 1? It needs to be added to materials list.

>We have added a breadboard to the material list.

Supplementary Figure 2 is never referred to in the protocol.

>We have added a reference to Supplementary Figure 2.

Figure 3a and Supplementary Figure 4 seem to be identical.

>It is a mistake. We have removed Supplementary Figure 4.

EWOD and PCB should probably be spelled out when first mentioned.

>We have made sure EWOD and PCB is spelled out when first mentioned.

Reviewer #2:
Manuscript Summary:
The authors report an education kit for demonstrating electrowetting droplet actuation. Compared to previously reported systems, the kit proposed in the present paper can be assembled in a relatively short period of time and with minimal training in electronics and soldering. More importantly, the proposed electronic circuit and components cost much less and provide the same functions. They demonstrated the effectiveness of this kit using three different experiments. This paper is generally well written and presents a useful education toolkit for electrowetting experiments. The method is technically sound and clearly presented. I do, however, have a few comments and suggestions that should be addressed before acceptance:

Major Concerns:
None.

Minor Concerns:
1. The kit is specifically for electrowetting droplet actuation. However, in the Introduction section the authors discuss general microfluidics technology. It is not clear if the papers they cited are also for electrowetting or for other microfluidic phenomena. Electrowetting is only

one of several key microfluidic phenomena.

> This is a very good point. A more pertinent term for our kit is digital microfluidics. We therefore changed electrowetting to "digital microfluidics". Digital microfluidics means the manipulation of droplet with electrode and is a more general term.

2. The abbreviation "EWOD" is first used in Line 83 without showing its full name "electrowetting-on-dielectric." The EWOD should be introduced in the Introduction section with its full name.

> We have made sure "EWOD" is spelled out when first used.

3. It will be useful if the authors can provide specifications of the proposed kit. For example, what kinds of liquid droplets can be used (material, size, viscosity, etc.)?

> We have performed an extensive test on the usable liquid droplet in terms of size, ionic concentration, PH value, viscosity and so on. The result is summarized in Table 2. The following reference is also added for glycerol water viscosity(Jean-Marc Busnel, et al., Electrophoresis 2005).

4. Scale bars should be added to Figures, or describe the dimension in the figure captions.

>We have made sure scale bar is added.

Reviewer #3:

Manuscript Summary:

The manuscript describes a simplified digital microfluidic/EWOD system to decrease barriers to participation in microfluidics. The authors claim this system could be used in education and community settings. The authors describe how to assemble the system using low-cost and off-the-shelf componentry. They then demonstrate how the system can be used to move a droplet, record droplet movement, and monitor luminescence. An atomizer humidification system is also included to minimize droplet evaporation. Overall, this system is a useful addition to the existing repertoire of open source digital microfluidic actuation systems.

Major Concerns:

The main claim, as given in the title, is that this platform is appropriate for education and community contexts. The authors even suggest (only in the abstract) that this could be used for undergraduate education. If the authors are going to argue that their system is specifically tailored for educational purposes, they should provide evidence to that end. This could include

outlining what learning objectives they envisage the platform can be used for, how long it takes an undergraduate with minimal training in electronics and soldering to assemble the kit and get it functional, and any data (anecdotal or otherwise) on student experiences with the platform.

>We have solicited 13 undergraduate students with no previous experience on soldering for ~ 2hr and the test result is included in the manuscript. Through the process, we have also identified the bottleneck process. This result is included in the Discussion.

The authors claim that the OpenDrop platform requires soldering of dozens of components (if not outright purchasing an assembled unit) and that their system, since it relies on fewer soldered components, is easier for someone with minimal training in electronics and soldering. However, their system requires soldering of 28 surface mounted components. Do they suggest that skill in soldering limits the number of components someone can solder, as it would seem if one can solder 28 components, they could solder dozens more?

> Also we may naively think soldering 28 components has the same difficulties as dozens more. To give the reviewer a rough idea. In a simple statistical model assuming one component, if the probability of success for one component is P, the cumulative success rate P^n for a n component system. Hence, the number of component critically affect the successful rate.

Indeed, the soldering is most critical process of success from our actual test result with 13 undergraduate students. In this revision, we have also deleted some component and minimized the number of components to 18. These core of the circuit consists of only 8 transistors and 8 resisters for droplet actuation. The other two light emitting diodes are used for fluorescence imaging.

The integration of an atomizer for humidity control is potentially important for minimizing droplet evaporation. The authors only present data suggesting that a droplet can move after 1 hr and how the humidity within the enclosure is controlled. The authors should provide data showing any changes in droplet volume between non-humidified and humidified incubation on the platform.

> We measured the droplet volume change as a result of atomizer. This result is included in Representative Results.

Minor Concerns:
Reference 2 is better served as Choi, K (2012) Digital Microfluidics Annu. Rev. Anal. Chem. 5, 413-440.

> Thanks for the suggestion. This is indeed a better reference. We have put this to replace Ref. 2. Now it appears as Ref 10 in the revision.

The use of microfluidics in STEM education should be discussed in the introduction. Some potential references to include are:

Rackus et al, (2019) "Learning on a chip:" microfluidics for formal and informal science education, Biomicrofluidics 13,4, 041501.

Fintschenko (2011) Education: a modular approach to microfluidics in the teaching laboratory, Lab Chip, 11, 3394.

Legge (2002) Chemistry under the microscope—Lab-on-a-chip technologies, J. Chem. Educ. 79, 2, 173.

> Thanks for the suggestion. These references are included and serves as key ones in the introduction. Discussion of STEM is also mentioned when Fintschenko's paper is mentioned.

In comparing the costs between different systems (DropBot v3 $5,500, OpenDrop v4 $1,000, the presented system $100) the authors should also compare functionality and application. The DropBot is designed for research applications while the OpenDrop overlaps with the educational/community-based context of the system presented in this manuscript. While the OpenDrop may cost 10x more, it also has far greater functionality and potential for use in an educational context, depending on what the learning objectives are.

> Thanks for the suggestion. We have included Table 1 for their comparison.

There are many typographical errors throughout the manuscript. One in particular is the term "fluorescent microscope." The microscope is not fluorescent but images fluorescence, thus the correct term is "fluorescence microscope."

> Thanks for the suggestion. We have corrected the typo.

The EWOD PCB is not included in the list of supplementary items. The authors motivate their work claiming that PCBs enable sharing of digital design files. Please stand by this and share your PCB file in the supplementary information.

>We will make sure PCB files are available.

The sentence in the abstract "The high-voltage switching is based on a high breakdown voltage, and the entire operation can be powered by batteries" is unclear. How is the switching based on a high breakdown voltage? It would be more accurate to say that the switching is based off a

MOSFET that can withstand high voltages.

> This is a typo. The high voltage switching is based a MOSFET.

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Sincerely,

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