Journal of Visualized Experiments
A Versatile Kit Based on Digital Microfluidics Droplet Actuation for Science Education
--Manuscript Draft--

Article Type:	Methods Article - JoVE Produced Video
Manuscript Number:	JoVE61978R1
Full Title:	A Versatile Kit Based on Digital Microfluidics Droplet Actuation for Science Education
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TITLE:

A Versatile Kit Based on Digital Microfluidics Droplet Actuation for Science Education

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KEYWORDS:

electrowetting, digital microfluidics, community driven microfluidics, chemical education, lab on a chip, education tool

SUMMARY:

We describe an educational kit that allows users to execute multiple experiments and gain hands-on experience on digital microfluidics.

ABSTRACT:

This paper describes an educational kit based on digital microfluidics. A protocol for luminol-based chemiluminescence experiment is reported as a specific example. It also has fluorescent imaging capability and closed humidified enclosure based on an ultrasonic atomizer to prevent evaporation. The kit can be assembled within a short period of time and with minimal training in electronics and soldering. The kit allows both undergraduate/graduate students and enthusiasts to obtain hands-on experience in microfluidics in an intuitive way and be trained to gain familiarity with digital microfluidics.

INTRODUCTION:

Microfluidics is a highly interdisciplinary field combing physics, chemistry, biology, and engineering for the manipulation of small volume of liquids ranging from femtoliter to microliters[1]. Microfluidics is also a very broad and active field; a Web of Science search returns nearly 20,000 publications and yet there is insufficient literature and review papers on the usage of microfluidics as educational tool[2]. There are two insightful, albeit outdated review articles by Legge and Fintschenko[3,4]. Legge introduces educators to the idea of a lab on a chip[3]. Fintschenko pointed out the role of microfluidics teaching lab in Science Technology Engineering Mathematics (STEM) education and simplified the philosophies into “teach microfluidics” and “use microfluidics”[4]. A more recent review by Rackus, Ridel-Kruse and Pamme in 2019 points out that in addition to being interdisciplinary in nature, microfluidics is also a very hands-on subject[2]. The hands-on activity related to the practice of microfluidics lends students to inquiry-based learning and makes it an engaging tool for science communication and outreach. Microfluidics indeed offers much potential for science education in both formal and informal settings and is also an ideal "tool" to enthuse and educate the general public about the interdisciplinary aspect of modern sciences.

Examples such as low-cost microchannel devices, paper microfluidics, and digital microfluidics are ideal tools for educational purposes. Among these platforms, digital microfluidics remains esoteric and peer-reviewed reports based on digital microfluidics are lacking[2]. Here we propose to use digital microfluidics as an educational tool for several reasons. First, digital microfluidics is very distinct from microchannel-based paradigm because it is based on manipulation of the droplets and usage of the droplets as discrete microvessels. Second, droplets are manipulated on relatively generic electrode-array platforms so digital microfluidics can be intimately coupled with microelectronics. Users can leverage on an extended set of electronic components, now highly accessible for do-it-yourself applications to electronically interface with droplets. Hence, we argue that digital microfluidics can let students to experience these unique aspects and be open-minded not overly to stick to microchannel-based low Reynold number microfluidics[1].

Briefly, the field of digital microfluidics is largely based on the electrowetting phenomena, which was first described by Gabriel Lippmann[5,6]. The recent developments were initiated by Berge in the early 1990s[7]. His key contribution is the idea of introducing a thin insulator to separate the conductive liquid from metallic electrodes to eliminate the problem of electrolysis. This idea has been termed as electrowetting on dielectric (EWOD). Subsequently, the digital microfluidics was popularized by several pioneering researchers[8,9]. Now a comprehensive list of applications for example, in clinical diagnostics, chemistry and biology, has been proven on digital microfluidics[10,11,12] and, therefore, plenty of examples are available for an educational setting. In particular, along the line of low cost, do-it-yourself digital microfluidics, Abdelgawad and Wheeler have previously reported low-cost, rapid prototyping of digital microfluidics[13,14]. Fobel et al., has also reported DropBot as an open source digital microfluidic control system[15]. Yafia et al., also reported a portable digital microfluidics based on 3D printed parts and smaller phone[16]. Alistar and Gaudenz have also developed the battery powered OpenDrop platform, which is based on the field effect transistor array and dc actuation[17].

Here, we present a digital microfluidics educational kit based on commercially sourced printed circuit board (PCB) that allows the user to assemble and get hands-on experience with digital microfluidics (**Figure 1**). Fee-for-service to create PCB from digital design files is widely available, and hence we think it is a viable low-cost solution for education provided that digital design files can be shared. Meticulous choice of components and system design is made to simplify the assembly process and make an interface with the user's intuitive. Hence, a one-plate configuration is used instead of a two-plate configuration to avoid the need for a top plate. Both the components and the test chemicals need to be easily available. For example, food wrap from the supermarket is used as the insulator in our kit.

To prove feasibility of our kit, we suggest a specific chemistry experiment based on chemiluminescence of luminol and provide the protocol. The hope is that visual observation of chemiluminescence can enthuse and excite students. Luminol is a chemical that exhibits a blue glow when mixed with an oxidizing agent such as H_2O_2 and is typically used in forensics to detect blood[18]. In our laboratory setting, potassium ferricyanide serves as the catalyst. Luminol reacts with the hydroxide ion and forms a dianion. The dianion subsequently reacts with oxygen from hydrogen peroxide to form 5-aminophthalic acid with electrons in an excited state, and relaxation of electrons from the excited state to the ground state results in photons visible as a burst of blue light.

We also report a fluorescent imaging experiment with a smart phone to demonstrate the integration of a light-emitting diode (LED) as an excitation light source. Finally, droplet evaporation is a problem in microfluidics but is rarely being addressed. (A 1 μL of water droplet is lost within 1 h from an open substrate[3].) We use an atomizer based on a high-frequency piezo transducer to convert water into fine mist. This creates a humidified environment to prevent droplet evaporation and demonstrates long-term (~1 h) droplet actuation.

[Place **Figure 1** Here]

[Place **Figure 2** here]

PROTOCOL:

1) Assembling the digital microfluidics kit

1.1) Solder the surface mount resistors, transistors, and light-emitting diodes onto the PCB board according to the schematics in **Figure 1b**.

1.2) Connect the output of the high-voltage power supply board to the PCB board with soldered components (**Figure 2** and **Supplementary Figure 1**).

1.3) Connect the battery to the voltage booster board to boost the voltage from 6 V to 12 V (**Figure 2** and **Supplementary Figure 1**).

1.4) Connect the high-voltage supply board to the voltage booster board to boost the voltage from 6 V to 12 V (**Figure 2** and **Supplementary Figure 1**).

1.5) Connect the humidity sensor to the microcontroller board. Connect the ultrasonic piezo atomizer and the atomizer driver board to the microcontroller board (**Figure 2** and **Supplementary Figure 1**).

1.6) Place the whole assembly into the acrylic enclosure of dimensions 23 cm x 20.5 cm x 6 cm.

1.7) Turn on the microcontroller with the code (**Supplementary Code**) and use the digital multimeter to measure the voltage of the EWOD electrode to make sure the output voltage is ~230 V. Adjust the variable resistor of the high-voltage supply board such that the output voltage is ~230 V (**Supplementary Figure 2**).

2) Preparation of insulator on the electrode array

2.1) Wear clean nitrile gloves. Use a micropipette to apply ~10 μL of 5 cSt silicone oil on the electrode area and use a finger to spread the silicone oil evenly on the electrode area. Note that the silicone oil serves as the filling between electrode and food wrap insulator and to avoid any airgap.

2.2) Cut a piece of food wrap with dimensions of approximately 2.5 cm x 4 cm and place it on top of the electrode. Use the micropipette to apply ~10 μL of 5 cSt silicone oil on the electrode area and use a finger to spread the silicone oil evenly. Note that the silicone oil serves as a hydrophobic layer on top of the insulator.

3) Chemiluminescence experiment based on luminol

3.1) Mix 0.25 g of luminol and 1.6 g of NaOH in 25 mL of deionized water in a beaker with a glass stirrer to obtain a solution.

3.2) Mix 20 mL of the solution from the previous step with 20 mL of 3% hydrogen peroxide.

3.3) Use a micropipette to place 2–5 μL of the luminol solution from the previous step on the target electrode.

3.4) Use a micropipette to place 10 μL of 0.1% w/w potassium ferricyanide on the electrode. Note that this is the droplet to be moved for electrowetting.

3.5) Turn on the microcontroller to move the 10 μL droplet of potassium ferricyanide to merge with the luminol.

4) Fluorescent imaging experiment

4.1) Cut a piece of semi-transparent tape with dimensions of ~1 cm x 1 cm. Place the semi-transparent tape between the excitation light-emitting diode and EWOD electrodes.

4.2) Attach the emission color glass filter on the camera of the smart phone with tape.

4.3) Mix 2.5 mg of fluorescein isothiocyanate in aqueous ethanol (3% w/w) solution.

4.4) Pipette ~10 μL of the solution from the previous step on one of the electrodes.

4.5) Turn on the microcontroller.

4.6) Use the smart phone to record a video of droplet actuation.

5) Long-term droplet actuation experiment with ultrasonic atomizer

5.1) Place 1 mL of water onto the ultrasonic atomizer. Note that the code is written to use a threshold feedback algorithm to maintain a humidity level over 90%.

5.2) Place a 10 μL droplet with a micropipette. Turn on the microcontroller and immediately close the lid of the enclosure.

5.3) Wait for ~1 h. Visually check droplet actuation.

REPRESENTATIVE RESULTS:

The droplet actuation is recorded with a smart phone. Representative results for chemiluminescence and fluorescent imaging are displayed in **Figure 2** and **Figure 3**. For the chemiluminescence experiment, the droplet of 10 μL ferricyanide is actuated to move and mix with pre-deposited 2 μL droplet on the target electrode as shown in **Figure 3**. The time period between successive movement is set to be 4 s, slow enough for easy observation. Note that the burst of blue light resulting from mixing luminol solution (with hydrogen peroxide) with potassium ferricyanide can be seen with the naked eye even under ambient light. For fluorescent imaging displayed in **Figure 4**, the experiment needs to be carried out in the dark. The semi-transparent tape serves as the diffuser to evenly distribute the excitation light onto the droplet. The emitted light from the fluorescence is filtered with a low-cost emission filter attached on the smart phone camera. This imaging scheme is simpler than the usual dichroic mirror based scheme in a typical benchtop fluorescence microscope. For a long-term (~1 h) experiment, successful droplet actuation can be observed as shown in **Figure 5a**. **Figure 5b** shows representative humidity data under the action of an ultrasonic atomizer. We also measure the droplet diameter with and without atomizer. Without atomizer, the droplet diameter shrinks from 4.0 mm to 2.2 mm and volume changes from 10 μL to 6 μL at room temperature and ambient relative humidity of ~57%. With atomizer, the droplet diameter shrinks from 4 mm to 3.1 mm and volume changes from 10 μL to 8 μL at room temperature and ambient relative humidity >90%.

[Place **Figure 3** here]

[Place **Figure 4** here]

[Place **Figure 5** here].

FIGURE AND TABLE LEGENDS:

Figure 1: Schematics of EWOD set up. (**a**) A microcontroller is used to provide a control sequence to the EWOD electrode. Also, the humidity is controlled. (**b**) Schematics of PCB layout. Electrodes, LED for fluorescent imaging, resistor, and field effect transistors (FET) are labeled. Scale bar of 1 cm is also shown.

Figure 2: Top view of the kit. Microcontroller board, high voltage supply board, EWOD PCB, humidity sensor, and atomizer are labeled.

Figure 3: Snapshot of droplet movement and chemical luminescence. At t = 12 s, mixing of luminol with potassium ferricyanide results in a visible burst of blue light. Scale bar of 1 cm is also shown.

Figure 4: Integration with fluorescent imaging capability. (**a**) Schematic of the setup. An LED serves as the light source for excitation. A semi-transparent clear office tape serves as a light diffuser. The emission filter is directly attached to the smart phone camera. (**b**) Fluorescent imaging of the droplet containing fluorescein isothiocyanate.

Figure 5: Droplet actuation under humidity control with ultrasonic atomizer. (**a**) Snapshot of droplet movement after 1 h. Scale bar of 1 cm is also shown. (**b**) Relative humidity versus time under the action of the ultrasonic atomizer. An arrow indicates the atomizer is off owing to the threshold algorithm. The threshold for relative humidity is set to 90%.

Table 1: Comparsion between Dropbot, OpenDrop, and our Educational kit.

Table 2: Range of liquid system, parameters, and working range tested on our kit.

Supplementary Figure 1: Wiring schematics. Microcontroller and high voltage power supply board are powered by a battery. All operation is orchestrated with micro controller board. The atomizer is activated by the driver board.

Supplementary Figure 2: High voltage switching circuit. A high voltage metal oxide semiconductor field effect transistor (MOSFET) with a resistor is used to switch EWOD electrode.

Supplementary Table 1: Cost estimation of components of our kit. The unit cost of components such as transistors, resistors, light emitting diode are estimated from the bulk price of a pack of 10 to 100 components. The cost excludes the custom acrylic enclosure.

Supplementary Code: Custom script to enable the actuation for the droplet movement and ultrasonic atomizer to humidify the droplet environment.

DISCUSSION:

The procedure described here allows the reader to assemble and test a working EWOD system for droplet actuation and gain hands-on experience with microfluidics. We intentionally avoid expensive components and chemical samples. Currently, one kit can be constructed for ~$130 with the most expensive component being optical color glass for fluorescent imaging and microcontroller excluding the custom acrylic enclosure (**Supplementary Table 1**). For such a cost, a fluorescent imaging capability and an active humidity environmental control based on atomizer is also included. (A typical fluorescence microscope costs more than ~$1,500[19], and even a low-cost digital fluorescence microscope costs $300.) These low costs make our kit practical for a large-scale educational setting. For comparison, the Dropbot currently costs ~$5,000[20] and the OpenDrop platform costs ~$1,000[2]. A summary of comparison of these platforms is given in **Table 1**.

[Place **Table 1** Here]

To evaluate the feasibility of usage of our educational kit, we have solicited 13 undergraduate students of assorted background. Their major includes physics, biology, chemical engineering, medicine, material science, mechanical engineering, and electrical engineering. We purposely try to avoid the situation that students come overly from electrical engineering and arrange only one student with major in electrical engineering. We have instructed them to solder components to the PCB and in the end test droplet actuation on our kit within 2 h. No student except one from electrical engineering has previous experience on soldering. In the end, we collect the statistics. The successful rate is 62%. We found out that soldering the surface mount component is the bottleneck process of successful assembly of the kit. The general guideline is as follows. Fintschenko pointed out that tools or experiments fall somewhere in the spectrum between a do-it-yourself boundary and the black box boundary. With increasing engineering experience on the side of the students, e.g., from electrical engineering background, more of the laboratory session can take on the do-it-yourself flavor. However, inexperienced students in terms of electronics skills such as those on chemistry, biology, and biochemistry can derive a benefit on the black box end of the spectrum with kits preassembled by instructors.

For reference, we also try to delineate the parameter range of liquid droplets that can be used. For the size, we have tested the maximal and minimal liquid volume to be 16 μL and 8 μL, respectively with nominal liquid volume of ~10 μL employed. We have limited our liquid to aqueous solution and avoid organic solvents to avoid corrosion of polymer food wrap insulator. We have also picked commonly available liquid systems such as table sugar and salt to cover a range of parameters such as ionic concentration, PH value, density, and viscosity. The result is summarized in **Table 2**. Among these tests, we have picked glycerol water mixture as a means to test maximal viscosity of droplets while keeping other physical properties such surface tension relative constant. We determine the maximal weight percentage of glycerol and corresponding viscosity to be ~40% and 3.5 cp[21]. The maximal working ionic concentration up to 1 M is tested with sodium chloride. The PH value is tested with acetate, citric acid, and KOH solution.

[Place Table 2 here]

Here, we briefly discuss the physics involved for droplet actuation. Using the electromechanical derivation, driving force as a function of frequency and droplet position can be derived based on the energy capacity stored in the system from differentiation of this energy term. A critical frequency, fc, can be calculated for each device geometry/liquid combination[21]. Below this frequency, the estimated force reduces to that predicted by the thermodynamic method. In this regime, the force acting on the droplet arises from charges accumulated near the three-phase contact line being electrostatically pulled toward the actuated electrode. Above the critical frequency, a liquid-dielectrophoretic force dominates to pull the droplet toward the activated electrode. 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.

In conclusion, the overall experiment is designed to give the reader a hands-on exposure to digital microfluidics. More specifically, the kit allows students to learn optics, electronics, and fluidics so this aspect is suitable for any lab course in electrical engineering and mechanical engineering at the senior level. Also, the specific chemiluminescence experiment can be employed in a chemistry or chemical engineering experimental course at the senior level. While the experiment described here is a simplified version of a real-life scenario, it can be extended in a straightforward way to other experiments. For example, one can couple a paper test kit and move the droplet to the paper to be adsorbed. We can also easily combine a microprocessor with other interactive I/O devices to provide more sophisticated digital control and programmability. We believe that the protocol here can also benefit non-professional enthusiasts to learn and apply electronics to further advance their knowledge of the field.

ACKNOWLEDGMENTS:

Y. T. Y. would like to acknowledge funding support from the Ministry of Science and Technology under grant numbers MOST 107-2621-M-007-001-MY3 and National Tsing Hua University under grant number 109Q2702E1. Mark Kurban from Edanz Group (https://en-author-services.edanzgroup.com/ac) edited a draft of this manuscript.

DISCLOSURES:

The authors have nothing to disclose.

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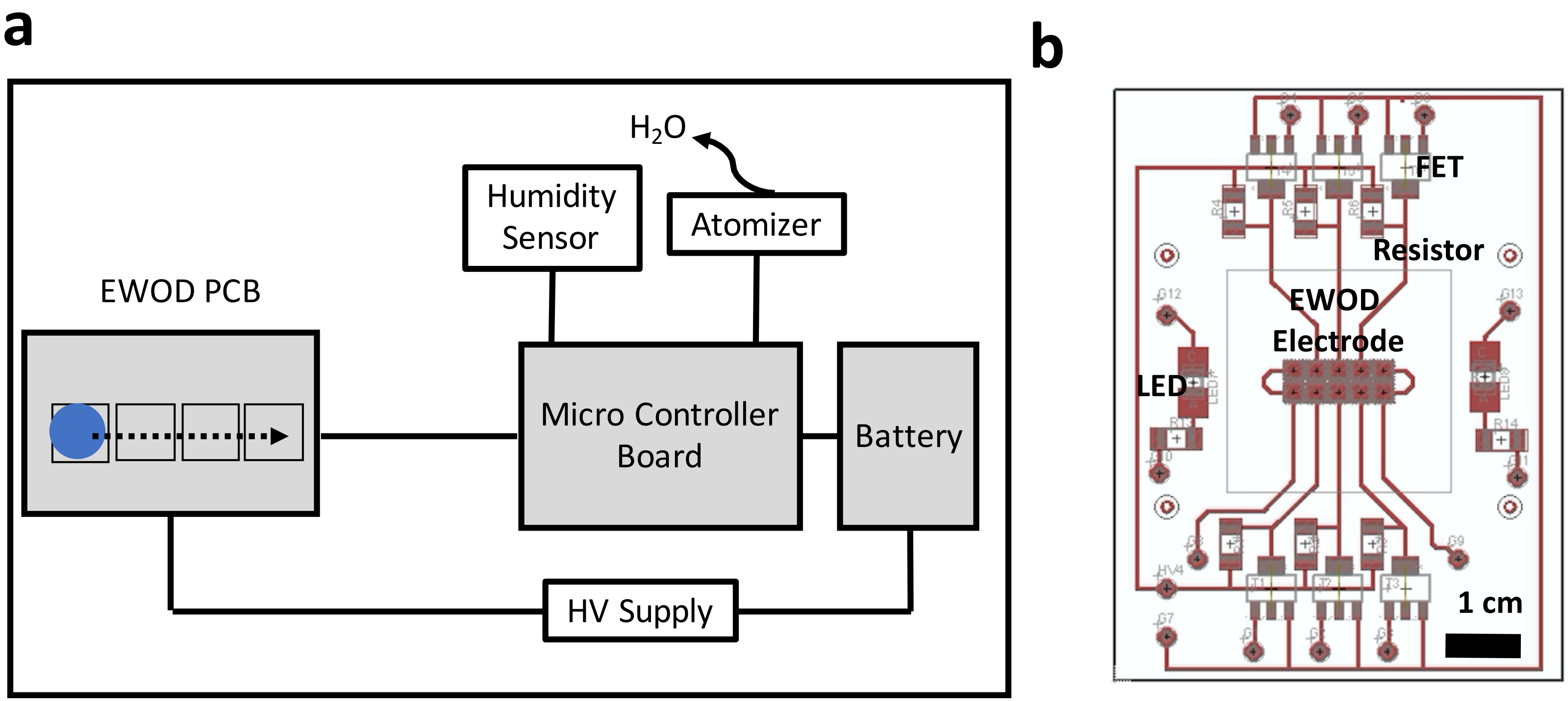

a
b
H_2O
Humidity Sensor
Atomizer
EWOD PCB
Micro Controller Board
Battery
HV Supply
FET
Resistor
EWOD Electrode
LED
1 cm

Figure 2

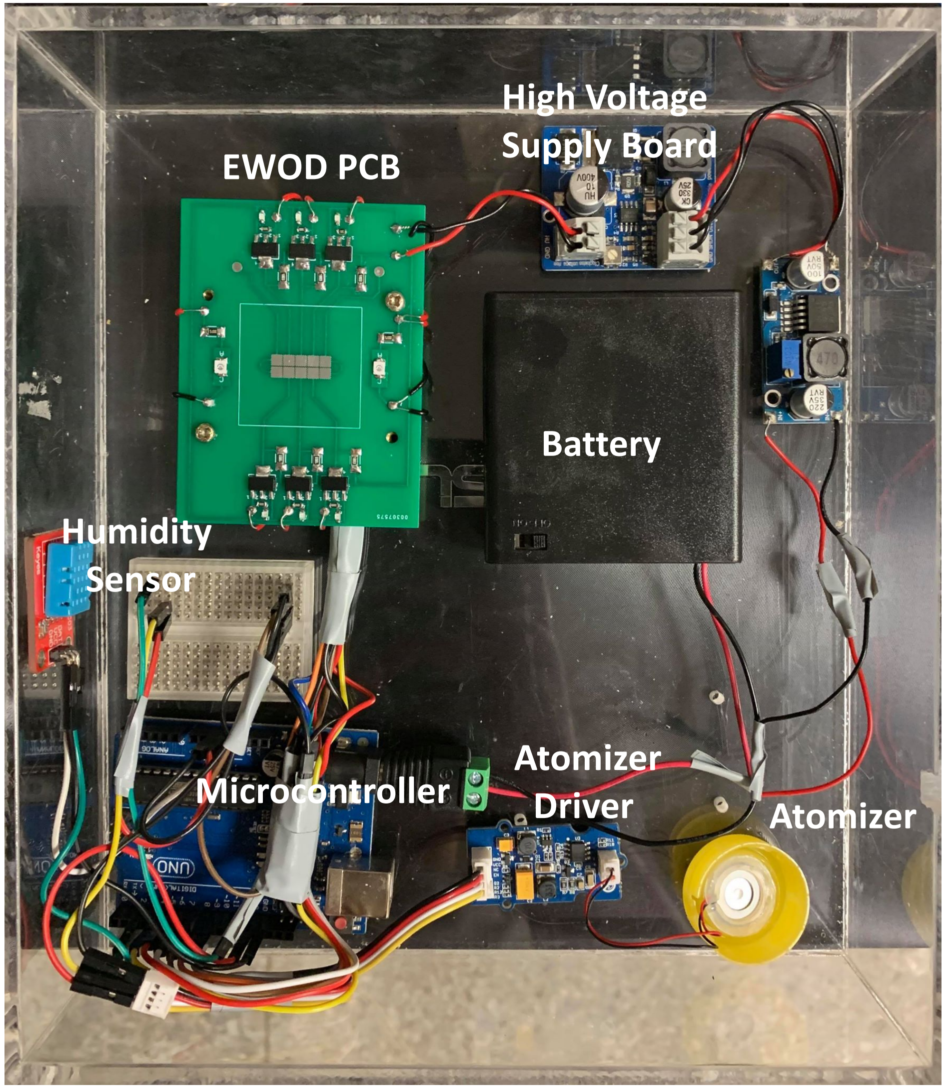

EWOD PCB
High Voltage
Supply Board
Battery
Humidity
Sensor
Microcontroller
Atomizer
Driver
Atomizer

Figure 3.

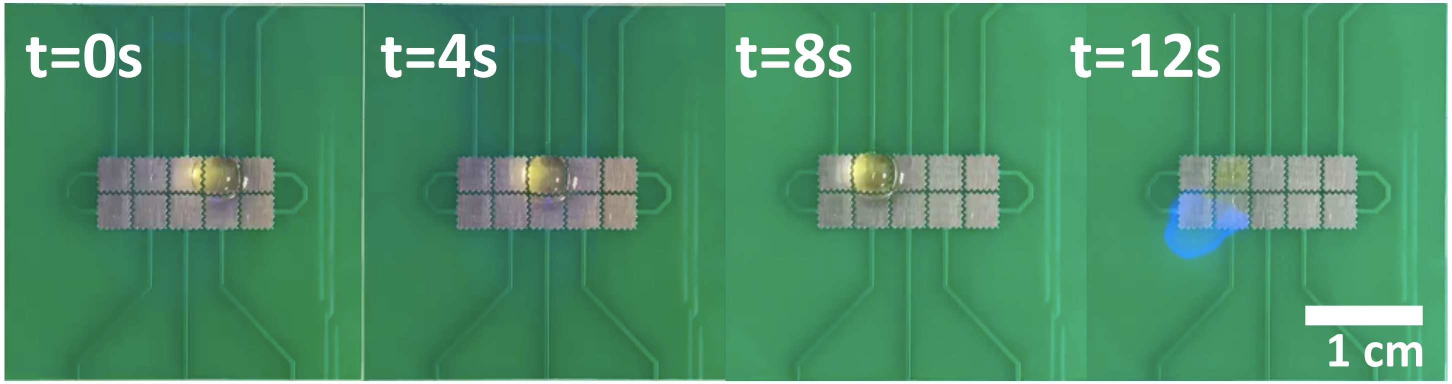


Figure 4a & 4b.

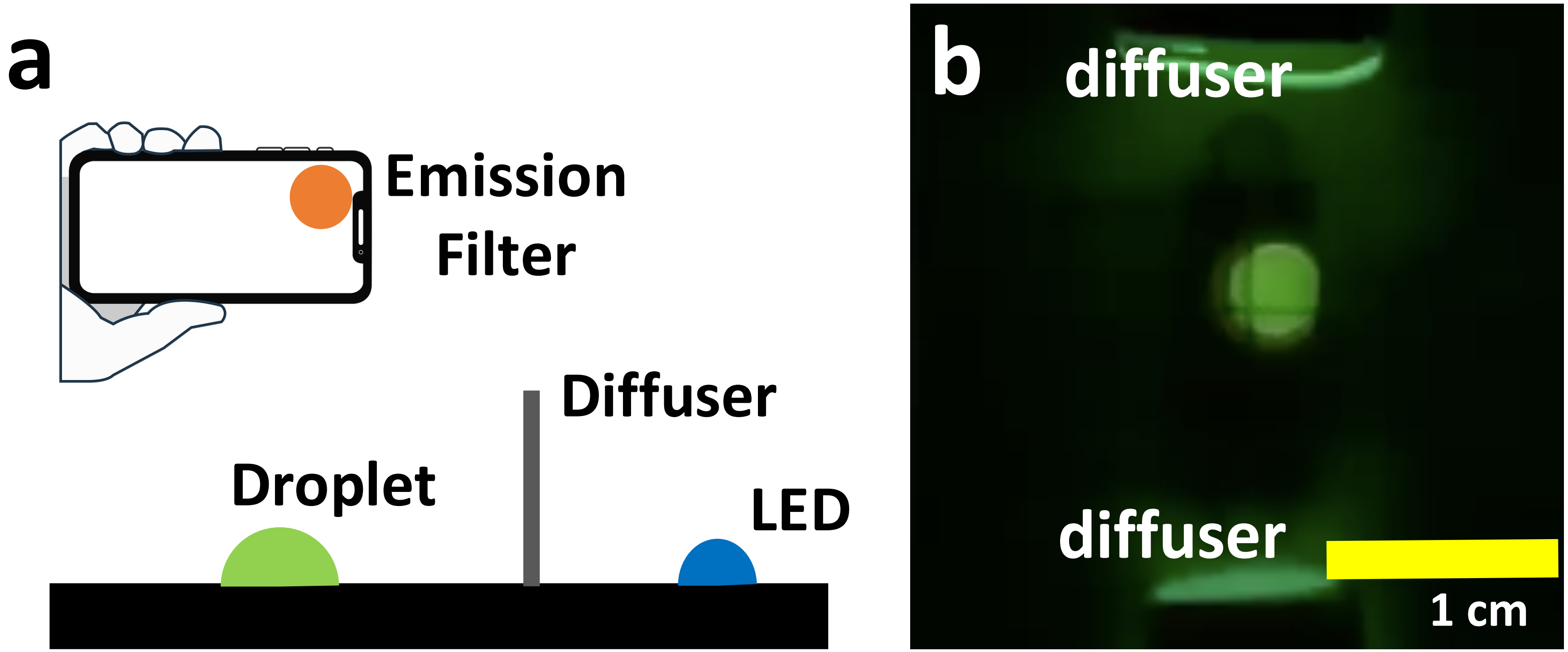
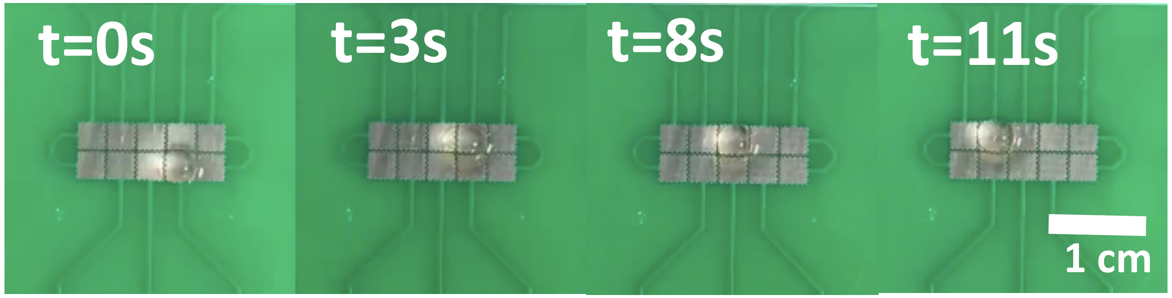
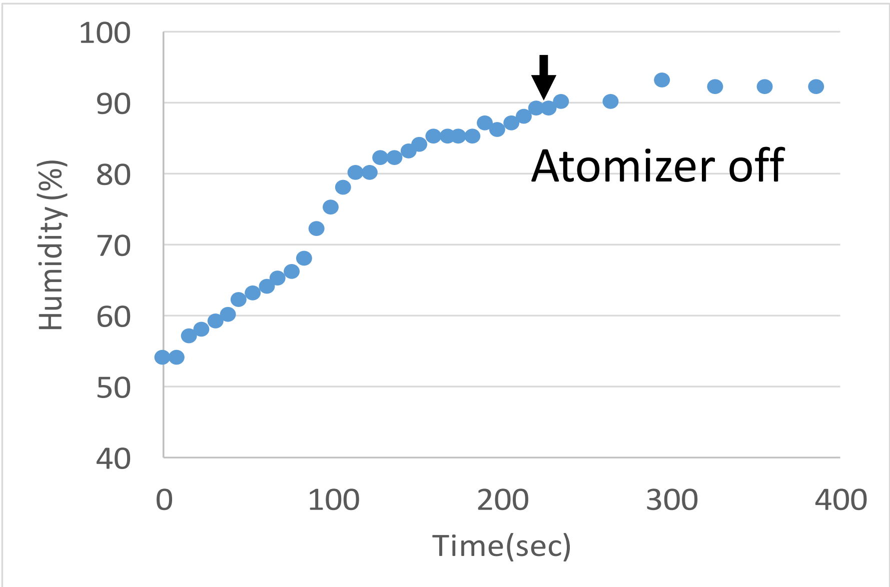

a
Emission
Filter
Diffuser
Droplet
LED
b
diffuser
diffuser
1 cm

Figure 5a & 5b.

a



b



Name of Material/ Equipment	Company	Catalog Number	Price
Acrylic enclosure	LOCAL vendor		30
Ardunion Uno	Arduino	UNO	30
acetic acid	Sigma Alrich	695092-100ML	70
Breadboard	MCIGICM	400tie	1.5
BSP89 H6327 Infineon MOSFET	Mouser	726-BSP89H6327	0.43
citrid acid	sigma Alrich	251275-100G	70
Color glass filter	Thorlabs	FGL 530	30
DHT11 temperature & humidity sensor	adafruit		11.25
Digital multimeter	Fluke	17B	191
Fluorescein isothiocyanate isomer I	sigma Alrich	F7250-50MG	50
Glycerol	Sigma Alrich	G9012-500ML	110
High voltage power supply for Nixe tube	Vaorwne	NCH6100HV	11.8
LM2596 voltage booster circuit			4
Luminol	Sigma Alrich	123072-5G	110
Pippet	Thermal Fisher		130
Printed circuit board	Local vender		6
Plastic food wrap	Kirkland	Stretch-tite	6
Potassium ferricynide	Merck	104982	130
1N Potassium hydroxide solution (1 mol/l)	Scharlau		30
Clear Office tape 3mm	3M Scotch		3
salt	Great Value Iodized Salt		6
Silicone oil (5Cst)	Sigma Alrich	317667-250ML	300
sucrose			6
Surface mount blue LED	oznium	3528	0.65
Surface mount resistor 180k Ohm	Balance World Inc		0.3

Surface mount resistor 510Ohm	Balance World Inc	0.3
Water atomizer	Grove	11

Comments/Description
23cm x 20.5 cm x 6cm
microcontroller board

4 cm x 7 cm, 400 Points Solderless Breadboard, a pack of 4
drain soure breakdown voltage 240V,on resistance 4.2 ohm

color glass filter for fluorescent imaging

50 mg price, fluorescent imaging

High voltage power max dc 235V
boost voltage from 5V to 12 V
5 g for $110
1- 10 ul
10 piece for $60
food wrap Plastic food wrap
1 kg
1 Liter
semi-transparent, used as diffuser for illumination

6 oz for $7 salt from supermarket

top hydrophobic layer & filling layer between electrode and
insulator
table sugar from any supermarket, 6 dollar per pound
Oznium 20 Pieces of PLCC-2 Surface Mount LEDs, 3528 Size SMD SMT LED - Blue
3mm x 6 mm 1watt

bias resistor for LED, 3mmx6mm 1watt
operating frequency 100 kHz supply votage 5V max 2W The kit comes with ultrasonic transducer
high voltage transistor

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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.

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

YA-TANG YANG
Associate Professor
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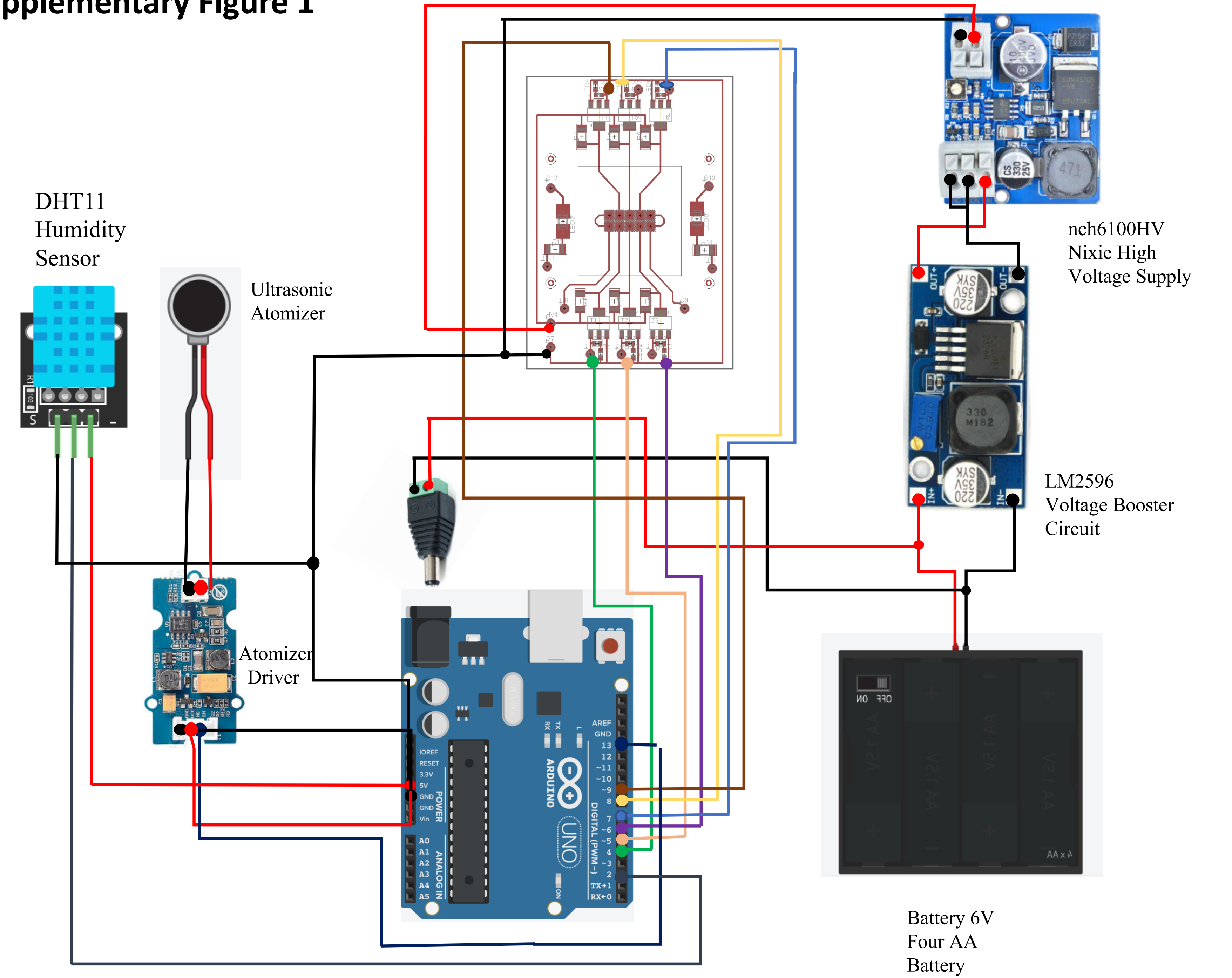
Component	Unit Price	Qty	Cost
Microcontroller board	30	1	30
Breadboard	1.5	1	1.5
HV MOSFET	0.43	6	2.58
Color glass filter	30	1	30
temperature & humidity sensor	11.25	1	11.25
High voltage power supply for Nixe tube	11.8	1	11.8
Voltage booster circuit	4	1	4
printed circuit board	6	1	6
Surface mount blue LED	0.65	2	1.3
Surface mount resistor 180k Ohm	0.3	6	1.8
Surface mount resistor 510Ohm	0.3	6	1.8
Water atomizer	11	1	11
		Total	113

Supplementary Figure 1



Supplementary Figure 2