Submission ID #: 61978
Scriptwriter Name: Domnic Colvin
Project Page Link: https://www.jove.com/account/file-uploader?src=18903338

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

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. Interview statements: Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

☒ Interview Statements are read by JoVE's voiceover talent.

4. Filming location: Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 12
Number of Shots: 28

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. We present a digital microfluidics educational kit based on commercially sourced printed circuit board that allows the user to get hands-on experience with digital microfluidics. This is a viable, low-cost solution for education, provided that digital PCB design files can be shared.

1.1.1. *Figure 1 a, then b.*

1.2. We propose to use digital microfluidics as an educational tool because droplets are manipulated on generic electrode-array platforms. Users can leverage on an extended set of electronic components, now highly accessible for do-it-yourself applications, to electronically interface with the droplets.

1.2.1. *2.3.1, 2.3.2.*

Introduction of Demonstrator on Camera

1.3. Demonstrating the procedure will be Yu Hao Guo, a graduate student from the Yang laboratory.

1.3.1. INTERVIEW: Author saying the above.

1.3.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera.

Protocol

2. Assembling the digital microfluidics kit and preparation of insulator on the electrode array

2.1. Begin by soldering the surface mount resistors, transistors, and light-emitting diodes onto the PCB board **[1-TXT]**. *Videographer: This step is difficult and important!*

2.1.1. Talent soldering the electrical components on PCB board. **TEXT: PCB- Power circuit board**

2.1.2. NOTE: Same as 2.1.1, different angle.

2.2. Connect the output of the high-voltage power supply board to the PCB board with the soldered components **[1-3]**, then connect the battery to the voltage booster board to boost the voltage from 6 volts to 12 volts **[4-6]**. *Videographer: This step is important!*

2.2.1. Added shot: Solder the power supply board to PCB

2.2.2. Talent connecting the PCB board to the power supply.

2.2.3. NOTE: may be an added shot here, not sure based on the author's notes. Maybe the previous shot was split in two.

2.2.4. Talent connecting battery to the voltage booster.

2.2.5. Added shot: Connecting 3 wires to booster board

2.2.6. Added shot: Solder to XL6009

2.3. Connect the humidity sensor **[1]**, the ultrasonic piezo atomizer, and the atomizer driver board to the microcontroller board **[2-3]**. *Videographer: This step is important!*

2.3.1. Talent connecting the humidity sensor to the microcontroller board. *Videographer focus on the humidity sensor and microcontroller board.*

2.3.2. Talent connecting the atomizer and atomizer driver board to the microcontroller board.

2.3.3. Added shot: A shot on full assembly

2.4. Turn on the microcontroller using the provided supplementary code **[1]**. Adjust the variable resistor of the high-voltage board **[2]** and use the digital multimeter to measure the voltage of the EWOD electrode **[3-TXT]**.

2.4.1. Talent turning on the microcontroller.

2.4.2. Talent adjusting the variable resistor of the high-voltage board.

2.4.3. Talent measuring voltage of EWOD electrode using multimeter. **TEXT: Output voltage should be ~230 V**

2.5. Wear clean nitrile gloves **[1]** and apply 10 microliters of 5 centistokes Silicone oil on the electrode area using a micropipette **[2]**. Spread the oil evenly on the electrode area using a finger **[3]**.

2.5.1. Talent wearing nitrile gloves.

2.5.2. Talent applying Silicone oil to the electrode area.

2.5.3. Talent spreading the oil.

2.6. Cut a piece of food wrap with dimensions 2.5 by 4 centimeters **[1]** and place it on top of the electrode **[2]**. Apply silicone oil on the electrode area using a micropipette **[3]** and spread it evenly **[4]**.

2.6.1. Talent cutting piece of food wrap.

2.6.2. Talent placing food wrap on the electrode area.

2.6.3. Talent applying silicone oil on the electrode area.

2.6.4. Talent spreading the oil using finger.

3. Chemiluminescence, fluorescent imaging, and long-term droplet actuation experiment

3.1. To perform a chemiluminescence experiment, place 2 to 5 microliters of luminol solution on the target electrode using a micropipette **[1]**. Place 10 microliters of 0.1% potassium ferricyanide on the electrode, which can be moved as a droplet for electrowetting **[2]**.

3.1.1. Talent placing luminol solution on the target electrode.

3.1.2. Talent placing potassium ferricyanide on the electrode.

3.2. Turn on the microcontroller so that the potassium ferricyanide droplet merges with the luminol **[1]**.

3.2.1. Talent turning on the microcontroller. NOTE: May have been split in 2 shots.

3.3. For fluorescent imaging, cut a 1 square centimeter piece of semi-transparent tape **[1]** and place it between the excitation light-emitting diode and EWOD electrodes **[2]**.

3.3.1. Talent cutting a piece of semi-transparent tape.

3.3.2. Talent placing the tape between light-emitting diode and EWOD electrodes.

3.4. Attach the emission glass filter on the camera of the smartphone with a tape **[1]** and place 10 microliters of the potassium ferricyanide solution on the electrodes **[2]**. Record the video of the droplet actuation using a smartphone **[3]**.

3.4.1. Talent attaching emission glass filter on the camera of smartphone.

3.4.2. Talent placing potassium ferricyanide on the electrode.

3.4.3. Talent recording the video of droplet actuation.

3.5. For long-term droplet actuation, place 1 milliliter of water onto the ultrasonic atomizer **[1-TXT]**. *Videographer: This step is important!*

3.5.1. Talent placing water on the atomizer. **TEXT: Maintain humidity level over 90%**

3.6. Place a droplet of potassium ferricyanide **[1]** and turn on the microcontroller **[2]**, then immediately close the lid of the enclosure **[3]**. Check the droplet actuation after an hour **[4]**.

3.6.1. Use shot 3.1.2

3.6.2. Talent turning on the microcontroller.

3.6.3. Talent closing the lid.

3.6.4. Talent checking the droplet actuation.

Results

4. The analysis of the droplet movement in fluorescent imaging and under the control of ultrasonic atomizer

4.1. For the chemiluminescence experiment, the droplet of ferricyanide is actuated to move and mix with the pre-deposited luminol droplet on the target electrode at 12 seconds **[1]**.

4.1.1. LAB MEDIA: Figure 3. *Video editor focus on the burst of blue light at t=12s.*

4.2. A schematic setup of an LED serving as the light source for excitation, a semi-transparent clear office tape as a light diffuser, and the emission filter directly attached to the smart phone camera is shown here **[1]**.

4.2.1. LAB MEDIA: Figure 4a.

4.3. Fluorescent imaging of the droplet containing fluorescein isothiocyanate in the dark is seen as a result of the semi-transparent tape serving as the diffuser to evenly distribute the excitation light **[1]**.

4.3.1. LAB MEDIA: Figure 4b.

4.4. For a long-term experiment, successful droplet actuation can be observed **[1]**. Representative humidity data under the action of an ultrasonic atomizer is shown here **[2]**.

4.4.1. LAB MEDIA: Figure 5a.

4.4.2. LAB MEDIA: Figure 5b.

Conclusion

5. Conclusion Interview Statements

5.1. This protocol can be used to develop an educational kit based on digital microfluidics. A luminol-based chemiluminescence experiment is reported as a specific example. The kit can be assembled within a short period of time and with minimal training in electronics.

5.1.1. *2.3.3.*

5.2. The simplified experiment described here can be extended to other experiments. For example, a paper test kit can be used by moving the droplet to the paper to be adsorbed. A microcontroller with interface logic circuit can also be added to provide more sophisticated digital control and programmability.

5.2.1. *3.6.1.*

5.3. This protocol can benefit non-professional enthusiasts to learn and apply electronics to further advance their knowledge of the field.

5.3.1. *3.6.4.*