Submission ID #: 62148
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Project Page Link: https://www.jove.com/account/file-uploader?src=18955188

Title: Open-Source Miniature Fluorimeter to Monitor Real-Time Isothermal Nucleic Acid Amplification Reactions in Resource-Limited Settings

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Author Questionnaire

1. Microscopy: Does your protocol demonstrate the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **N**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **Y**

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**.

☒ Interviewees wear masks until the videographer steps away (≥6 ft/2 m) and begins filming. The interviewee then removes the mask for line delivery only. When the shot is acquired, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

3. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **41**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Jackson Coole**: This method allows individuals without electronics expertise to build instruments such as this fluorimeter for isothermal nucleic acid amplification and detection, which is critical for molecular diagnostics **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

REQUIRED:

1.2. **Alex Kortum**: The main advantage of this technique is that the system can be completely assembled from commercially available materials and open-source software at a low cost **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

OPTIONAL:

1.3. **Kathryn Kundrod**: This fluorimeter can be used with multiple isothermal amplification methods. This is important, because isothermal amplification methods are increasingly used for the detection of a wide range of infectious and inherited diseases **[1]**.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Protocol

2. Optical Housing Assembly

2.1. To assemble the optical housing, place a 3/16-inch-long 4-40 threaded insert into the hole on the top of the Optics_Enclosure_Bottom.stl piece **[1]** and place a 1/4-inch-long 4-40 threaded insert into all of the other holes of the piece **[2]**.

2.1.1. WIDE: Talent placing insert into hole

2.1.2. Talent placing insert(s) into other hole(s)

2.2. Insert the sensor test board into the top cavity of the housing, with the five pins facing toward the top and closest to the center axis of the device, and secure the test board with a 3/16-inch-long 4-40 screw **[1]**.

2.2.1. Talent placing board into top cavity and securing board NOTE: 2.2.2 was shot with 2.2.1.

~~2.2.2. Talent securing board~~

2.3. Place one of the 20-millimeter focal length lenses in the section below the sensor test board with the convex side facing toward the bottom of the device and away from the test board **[1]**.

2.3.1. Talent placing lens into section below test board

2.4. To create the first configuration, place the long pass filter into the next section below the 20-millimeter focal length lens **[1]**. To create the second configuration, place two yellow emission filter foils into the section below the lens **[2]**.

2.4.1. Long pass filter being placed

2.4.2. Foils being placed

2.5. To create the first configuration, place the dichroic mirror into the diagonal section near the center of the encasement while observing the filter orientation specified by

the manufacturer **[1]**. To create the second configuration, place the beam splitter into the diagonal section **[2-TXT]**.

2.5.1. Mirror being placed

2.5.2. Beam splitter being placed **TEXT: No specific orientation needed for beam splitter**

2.6. Place a second 20-millimeter focal length lens into the section below the dichroic mirror or beam splitter, depending on configuration, with the convex side pointing toward the top of the device **[1]**.

2.6.1. Talent placing lens

2.7. To create the first configuration, place the excitation filter in the section to the right of the dichroic mirror, making sure the arrow points toward the dichroic mirror **[1]**. To create the second configuration, place one blue excitation filter foil into the section to the right of the beam splitter **[2]**.

2.7.1. Talent placing filter

2.7.2. Talent placing foil

2.8. Place the 15-millimeter focal length lens to the right of the excitation filter with the convex side facing the dichroic mirror **[1]** and place an LED into the remaining section of the print with the LED facing toward the dichroic mirror or beam splitter, depending on the configuration **[2]**.

2.8.1. Talent placing lens to right of filter

2.8.2. Talent placing LED

2.9. Make sure that the two wires leading from the LED are inserted into the recessed channels so that the print will close tightly **[1]** and repeat the setup for the other side of the 3D printed piece **[2]**.

2.9.1. Wires being checked *Videographer: Important step*

2.9.2. Shot of parts assembled on other side *Videographer: Important step*

2.10. Then place the extruded portions of the top half of the encasement into the recessed grooves of the bottom half of the encasement to close the empty side of the piece with the optical components **[1]** and secure the parts together with 3/8-inch-long 4-40 screws **[2-TXT]**.

2.10.1. Talent placing pieces into recessed grooves/closing piece

2.10.2. Talent inserting screws **TEXT: Caution: If parts not completely closed, stray excitation light can escape**

3. Electronics and Touchscreen Assembly

3.1. To assemble the electronics and touchscreen, connect the two mini breadboards **[1]** and place the microcontroller onto one of the breadboards, ensuring that the microUSB port of the microcontroller faces outward **[2]**.

3.1.1. WIDE: Talent connecting breadboard(s)

3.1.2. Talent placing microcontroller onto breadboard with USB port facing outward

3.2. To connect the LED modulation, connect the CTL (C-T-L) pin of the LED-plus driver to a digital pin of the microcontroller **[1]** and the LED-minus pin of the LED driver to a GND (G-N-D) pin of the microcontroller **[2]**.

3.2.1. CTL pin being connected to LED+ pin NOTE: Oral slate

3.2.2. LED- pin being connected to GND pin

3.3. Remove the plastic covers on the back of the breadboards **[1]** and press the adhesive backing of the breadboards to the 3D printed part to attach the combined breadboards to the inside of the back portion of the LCD_Screen_Holder.stl printed piece **[2]**.

3.3.1. Talent removing cover(s)

3.3.2. Talent pressing backing to 3D printed part

3.4. Secure the LCD screen holder with the assembled breadboards inside the optical enclosure with one-inch-long 4-40 screws **[1]**.

3.4.1. Holder being secured *Videographer: Important step*

3.5. To connect the LED power supply, connect the LED-positive pin of the LED driver to the positive wire of the first LED **[1]** and connect the negative wire of the first LED to the positive wire of the second LED on the breadboard **[2]**. Connect the negative wire of the second LED to the LED-minus pin of the LED driver **[3]**.

3.5.1. Talent connecting pin to positive wire *Videographer: Important step* NOTE: Step 3.5 was filmed in 4K, macro with audio slate

3.5.2. Talent connecting negative wire to positive wire *Videographer: Difficult step*

3.5.3. Talent connecting negative wire to pin *Videographer: Difficult step*

3.6. To connect the LED driver power supply, use a barrel jack to two-pin adapter to connect the positive and negative wires of the 10-volt power supply to the VIN (vin)-plus and VIN-minus pins of the LED driver, respectively **[1]**.

3.6.1. Positive and/or negative wires being connected

3.7. To connect the sensor test board power supply and data transfer, use a 4-pin female-to-male jumper wire to connect the SCK (S-C-K), SDA (S-D-A), VDUT (V-D-U-T), and GND pins on the light-to-digital sensor test boards to the mini breadboard through the gap in the top right of the LCD Holder print **[2]**.

3.7.1. Talent using jumper wire to connect pins to test boards

3.8. On the breadboard, confirm that the 3.3-volt pin of the microcontroller and the VDUT pin of both test boards **[1]**, the GND pin of the microcontroller and GND pin of both test boards **[2]**, the analog 4 pin of the microcontroller and the SDA pin of both test boards **[3]**, and the analog 5 pin of the microcontroller and the SCK pin of both test boards are all connected **[4]**.

3.8.1. Shot of pins *Videographer: Difficult step; Videographer/Video Editor: shot will be used again; Video Editor: please emphasize 3.3 V pin-VDUT pin connection* NOTE: Step 3.8 was filmed in 4K, macro with audio slate

3.8.2. Use 3.8.1. *Video Editor: please emphasize GND-GND pin connection*

3.8.3. Use 3.8.1. *Video Editor: please emphasize A4-SDA pin connection* NOTE: Second wire in 3.8.3a and 3.8.4a

3.8.4. Use 3.8.1. *Video Editor: please emphasize A5-SCK pin connection*

3.9. Use four M2.5 (M-two-point-five) screws to secure the single-board computer onto the LCD screen holder with the HDMI and power adapter ports of the single-board computer facing upward and the single-board computer centered on the 3D printed part **[1]**.

3.9.1. Screws being inserted/board being secured *Videographer: Important step; Video Editor: please emphasize ports and computer when mentioned if possible/appropriate*

3.10. Then connect the touchscreen display to the single-board computer according to the touchscreen instructions **[1]** and connect the HDMI port of the single-board computer to the HDMI port of the touchscreen **[2]**.

3.10.1. Talent connecting display to computer *Videographer: Important step*

3.10.2. Talent connecting ports *Videographer: Important step*

4. Real-Time Fluorescence Data Recording

4.1. To record the real-time fluorescence data, after the heat block has been turned on and has reached the appropriate temperature **[1]**, power on the single-board computer **[2]** and use a microUSB-to-USB cable to connect the single-board computer to the microcontroller **[3]**.

4.1.1. WIDE: Talent turning on/checking heat block temperature *Videographer: Important step*

4.1.2. Talent powering on computer *Videographer: Important step*

4.1.3. Talent connecting computer to microcontroller *Videographer: Important step*

4.2. Open the provided Python script on the touchscreen **[1]** and change the measurement_time **[2]**. Change the variable output File path to the name of the data file the program generates **[3-TXT]** and change the serial Port variables to the desired values **[4]**.

4.2.1. Talent opening script

4.2.2. SCREEN: 62148_1: 00:00-00:07

4.2.3. SCREEN: 62148_1: 00:07-00:13 **TEXT: Filename must end in .xlsx**

4.2.4. SCREEN: 62148_1: 00:13-00:23

4.3. Place two PCR tubes containing the reactions to be monitored into the heat block **[1]** and place the fluorimeter onto the heat block with the PCR tubes centered between the four pegs extruding from each optical channel **[2]**.

4.3.1. Talent placing tube(s) into block *Videographer: Important step*

4.3.2. Talent placing fluorimeter onto block *Videographer: Important step*

4.4. After confirming that the 3D printed fluorimeter is attached, plug in the power supply adapter for the LEDs **[1]** and start the Python program. A graphical user interface will appear on the LCD screen for measuring the real-time fluorescence in both PCR tubes **[2]**.

4.4.1. Talent plugging in power supply

4.4.2. SCREEN: screenshot_2: 00:00-00:32 *Video Editor: please speed up*

4.5. At the end of the experiment, view the measurements in the output data file saved in the user-defined location **[1-TXT]**.

4.5.1. SCREEN: screenshot_3: 00:00-00:11 **TEXT: Click Stop Acquisition to end measurements early if necessary**

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see? Please list 4 to 6 individual steps.

2.9., 3.4., 3.9., 3.10., 4.1., 4.3.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success? Please list 1 or 2 individual steps from the script above.
3.5., 3.8. The most difficult aspect of this procedure would be ensuring the electronics are assembled correctly. For this, we follow the electrical diagram shown in Figure 2K and test if connections are correct by running the provided software before finishing assembly.

Results

5. Results: Representative Miniature Fluorimeter Measurements

5.1. Once assembled, the fluorimeter performance can be validated by measuring the fluorescence from a dilution series of FITC (FIT-sea) dye **[1]**.

5.1.1. LAB MEDIA: Figure 3A

5.2. In this representative analysis, both channels of the fluorimeter showed a linear response across the desired range **[1]**.

5.2.1. LAB MEDIA: Figure 3A *Video Editor: please sequentially emphasize black and blue data lines*

5.3. Shown here are the baseline-subtracted fluorescence **[1]** of the recombinase polymerase amplification-positive **[2]** and the negative-control reactions of a standard commercial kit measured on the second configuration of the fluorimeter **[3]**.

5.3.1. LAB MEDIA: Figure 3B
5.3.2. LAB MEDIA: Figure 3B *Video Editor: please emphasize blue data line*
5.3.3. LAB MEDIA: Figure 3B *Video Editor: please emphasize black data line*

5.4. Real time fluorescence measurements of a custom reverse transcription loop-mediated isothermal amplification reaction for SARS (sars)-Covid-2 RNA on the first configuration of the fluorimeter shows that amplification occurs as expected over a clinically-relevant range of RNA copy numbers **[1]**.

5.4.1. LAB MEDIA: Figure 3C *Video Editor: please sequentially emphasize pink, green, and blue data lines*

Conclusion

6. Conclusion Interview Statements

6.1. **Rebecca Richards-Kortum**: At a time when global supply chains are at their most fragile, open-source equipment like this fluorimeter have the potential to reduce pandemic-related health inequities **[1]**.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera