## Open-Source Miniature Fluorimeter to Monitor Real-Time Isothermal Nucleic Acid Amplification Reactions in Resource-Limited Settings
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1   **TITLE:**
2   Open-Source Miniature Fluorimeter to Monitor Real-Time Isothermal Nucleic Acid Amplification
3   Reactions in Resource-Limited Settings
4
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24  **KEYWORDS:**
25  open-source, fluorimeter, isothermal amplification, nucleic acid amplification test, low-resource
26  setting, SARS-CoV-2 detection
27
28  **SUMMARY:**
29  Detailed instructions are provided to build an open-source, modular fluorimeter that is
30  compatible with many low-cost heaters to perform real-time, quantitative isothermal nucleic
31  acid amplification.
32
33  **ABSTRACT:**
34  Traditional methods to detect and quantify nucleic acids rely on polymerase chain reaction (PCR)
35  and require the use of expensive thermocyclers with integrated fluorescence detection of
36  amplicons. Isothermal nucleic acid amplification technologies eliminate the need for thermal
37  cycling; however, fluorescence-based detection of products is still required for real-time,
38  quantitative results. Several portable isothermal heaters with integrated fluorescence detection
39  are now commercially available; however, the cost of these devices remains a significant barrier
40  to widespread adoption in resource-limited settings. Described here is a protocol for the design
41  and assembly of a modular, low-cost fluorimeter constructed from off-the-shelf components.
42  Enclosed in a compact 3D printed housing, the fluorimeter is designed to be placed atop a
43  commercially available heat block holding a PCR tube. The fluorimeter described here was
44  optimized to detect fluorescein isothiocyanate (FITC) dye, but the system can be modified for use

45  with dyes commonly used as reporters in real-time nucleic acid amplification reactions. Clinical
46  applicability of the system is demonstrated by performing real-time nucleic acid detection with
47  two isothermal amplification technologies: recombinase polymerase amplification (RPA) for
48  detection of positive control DNA provided in a commercial kit and reverse transcription loop-
49  mediated isothermal amplification (RT-LAMP) for detection of clinically meaningful levels of
50  SARS-CoV-2 RNA.
51
52  **INTRODUCTION:**
53  Isothermal amplification technologies are widely used for detection of nucleic acids. Compared
54  to traditional PCR approaches that require thermocycling, isothermal amplification allows nucleic
55  acid amplification to occur at a single temperature, thus enabling faster time-to-results and
56  better tolerance of inhibitors[1,2]. Another key benefit of isothermal amplification is the reduced
57  instrumentation complexity. Most isothermal amplification reactions only require a heat block
58  and a detection modality—either real-time detection via fluorescence monitoring or endpoint
59  detection, for example by lateral flow or gel electrophoresis[3,4]. Real-time fluorescence detection
60  is accomplished through the detection of fluorescence produced by intercalating dyes that
61  activate in the presence of double-stranded DNA or quenched fluorescent probes that activate
62  in the presence of specific double-stranded DNA sequences.
63
64  While commercially available benchtop isothermal fluorimeters exist, many lack customization
65  for assay implementation. For example, many devices require specific or company-provided
66  consumables, recommend preferred vendors, or utilize proprietary software to obtain advertised
67  results. Most of these systems cost over $5,000 USD, representing a significant barrier for
68  widespread use in resource-limited settings. In addition, users in low-resource settings face
69  challenges to maintain equipment designed for high-resource settings due to harsh
70  environmental conditions, weak supply chains for spare parts, and specialized tools required for
71  maintenance and repair[5]. To meet this need, described here is the design and assembly of a
72  modular and low-cost fluorimeter constructed from off-the-shelf components enclosed in a
73  compact 3D printed housing (**Figures 1A–C**) with two optional configurations. The first
74  configuration of this device uses commercially available glass filters and a dichroic mirror to block
75  excess background light and has a total cost of assembly of $830 USD. While these filters are
76  commonly used in fluorescence-based imaging systems, replacing expensive high-grade optical
77  filter foils has previously been shown to allow for nucleic acid detection[6]. The second
78  configuration of the fluorimeter incorporates these inexpensive filters and replaces the dichroic
79  mirrors with φ½" beam splitters, reducing the overall cost of the system from $830 to $450 USD.
80
81  Representative images of the assembly are shown for the first configuration in **Figure 1** and
82  **Figure 2**, but analogous images for the second configuration can be found in **Supplemental File**
83  **6**. To avoid the need for specialized optical alignment, the optical system has designated areas to
84  place each optical component and can be made with a relatively low-end 3D printer, allowing for
85  widespread use of the design. The only differences in construction and assembly for the two
86  configurations are the files used for 3D printing and the optical components placed into the
87  enclosure. The external dimensions of the 3D printed enclosure for both systems are the same.
88  A cost comparison of the two systems is shown in **Table 1**.

89

90    As shown in **Figure 1A**, to maintain a small form factor, the fluorimeter consists of ϕ½" (~12.5
91    mm) optics, coupled with compact illumination and detection that are placed to measure signal
92    through the top of the PCR tube. The system in **Figure 1** is designed to detect dyes with peak
93    excitation and emission wavelengths near 490 nm and 525 nm, respectively, including FITC and
94    closely related dyes such as SYBR and SYTO-9, which are commonly used as reporters in real-time
95    nucleic acid amplification reactions[7,8]. The excitation source, optical filters, and detector can
96    readily be substituted for components compatible with different fluorescent dyes as desired.
97    Nucleic acid amplification reactions are typically performed in PCR tubes, and the fluorimeter is
98    designed to be placed atop any commercially available heat block that holds PCR tubes (**Figure
99    1D**) allowing for real time monitoring of isothermal reactions. Appropriate heat blocks are
100   available in most biomedical laboratories and can be purchased for less than $500 USD.

101

102   The use of single-board computers to provide a low-cost, point of care alternative for controlling
103   imaging technologies has been previously demonstrated[9]. Building off that work, in this protocol
104   a single-board computer-powered graphical user interface (**Figure 1D**) is used to facilitate real-
105   time data logging and display of results at the point of care, eliminating the need for a laptop
106   computer to process or visualize data. Fluorescence measurements were transferred through $I^2C$
107   protocol from the light sensors to a microcontroller, and then made available to the single-board
108   computer through serial communication. Electrical connections for illumination and data transfer
109   were provided through simplified wiring and soldering on miniaturized breadboards, negating
110   the need for specialized printed circuit boards (PCBs). The software required to run the
111   fluorimeter is available through open-source software frameworks and the code required to run
112   the device is provided in the **supplemental coding files**. The complete fluorimeter can be
113   assembled for between $430 to $830 USD, and results show that it provides accurate and reliable
114   fluorescence measurements to monitor real-time isothermal amplification of nucleic acids.

115

116   **PROTOCOL:**

117

118   **1. Preparation steps: 3D printing and soldering**

119

120   NOTE: The optical system described in this protocol is designed for a standard dry block heater.

121

122   1.1.    To create the first configuration, 3D print the CAD files provided as **Supplemental Files 1**,
123   **2**, and **3** respectively:

124

125   1.2.    To create the second configuration, 3D print the CAD files provided as **Supplemental Files
126   3, 4**, and **5**, respectively:

127

128   NOTE: These parts are designed to be printed with supports. In this guide, a black polycarbonate
129   filament is used that can maintain its form after being subjected to temperatures up to 110 °C.
130   In general, any material that can be heated to the temperature of the desired isothermal reaction
131   without significant deformation can be used. To minimize the effect of internal reflections and
132   ambient light interference, a material that is black or another dark color is recommended.

133

134    1.3.    Prepare the two light-to-digital sensor evaluation modules for parallel monitoring of two
135    samples. On one of the sensor test boards, remove the R4 resistor, and solder a jumper wire from
136    the right pad of the R4 area on the PCB to the top pad in the R1 area on the PCB. This will change
137    the $I^2C$ address of the sensor, thus allowing simultaneous measurement of both sensors.

138

139    NOTE: The sensor used consists of two PCBs: a USB adapter board and a sensor test board
140    containing the light sensor; only the sensor test board is needed for this device.

141

142    1.4.    Solder wires to each of the two Light Emitting Diodes (LEDs). Connect a red wire (positive)
143    to the pad labeled "1" on the LED and a black wire (negative) on the pad labeled "2" on the LED.
144    Apply a thin layer of thermal adhesive on the back of the LED, place the LED on the top of an end
145    cap, and wait until the thermal adhesive cures. On the other side of the end cap, add a heat sink.

146

147    NOTE: When testing the LEDs before they are sealed in the enclosure, ensure to wear a proper
148    blue light blocking eye protection.

149

150    1.5.    To create the second configuration, cut two 1/4-inch diameter circles from a blue
151    excitation foil sheet and four 1/4-inch diameter circles from a yellow emission foil sheet with
152    scissors or a razor blade.

153

154    1.6.    Press an M2.5 hex shaped insert into each of the four holes on the slanted portion of the
155    'LCD_Screen_Holder.stl' part.

156

157    **2. Optical assembly**

158

159    2.1.    Place a 3/16-inch-long 4-40 threaded insert into the hole on the top of the
160    'Optics_Enclosure_Bottom.stl' part. Place a 1/4-inch-long 4-40 threaded insert into all other
161    holes of the 3D printed part as shown in **Figure 2A**.

162

163    2.2.    Insert the sensor test board into the top cavity of the housing, with the five pins facing
164    towards the top and closest to the center axis of the device. Secure with a 3/16-inch-long 4-40
165    screw through the hole on the sensor test board (**Figure 2B**).

166

167    2.3.    Place one of the 20 mm focal length lenses in the section below the sensor test board
168    with the convex side facing towards the bottom of the device and away from the test board
169    (**Figure 2C**).

170

171    2.4.    To create the first configuration, place the long pass filter into the next section below the
172    20 mm focal length lens (**Figure 2D**) placed in the previous step. To create the second
173    configuration, place two yellow emission filter foils into the section below the lens.

174

175    2.5.    To create the first configuration, place the dichroic mirror into the diagonal section near
176    the center of the encasement while observing the filter orientation specified by the manufacturer

177 (**Figure 2E**). To create the second configuration, place the beam splitter into the diagonal section.
178 No specific orientation is needed for the beam splitter.
179
180 2.6.    Place a second 20 mm focal length lens into the section below the dichroic mirror (or
181 beam splitter, depending on configuration) with the convex side pointing towards the top of the
182 device (**Figure 2F**).
183
184 2.7.    To create the first configuration, place the excitation filter in the section to the right of
185 the dichroic mirror, making sure the arrow points towards the dichroic mirror (**Figure 2G**). To
186 create the second configuration, place one blue excitation filter foil into the section to the right
187 of the beam splitter.
188
189 2.8.    Place the 15 mm focal length lens to the right of the excitation filter with the convex side
190 facing the dichroic mirror (**Figure 2H**).
191
192 2.9.    Place an LED into the remaining section of the print, with the LED facing towards the
193 dichroic mirror (or beam splitter, depending on configuration). Ensure the two wires leading from
194 the LED are inserted into the recessed channels so that the print will close tightly.
195
196 2.10.   Repeat steps 2.3–2.9 for the other side of the 3D printed part (**Figure 2I**).
197
198 2.11.   Close the empty side of the print on top of the print with the optical components by
199 placing the extruded portions of the top half of the encasement into the recessed grooves of the
200 bottom half of the encasement. Secure the two printed parts together with 3/8-inch-long 4-40
201 screws (**Figure 2J**).
202
203 NOTE: If the two printed parts are not completely closed, stray excitation light can escape from
204 the optical housing. Ensure proper blue light blocking eye protection is worn until a proper seal
205 is achieved. Reseal the enclosure until no excess light escapes.
206
207 **3. Electronics and touchscreen assembly**
208
209 3.1.    Connect the two mini breadboards together, and then place the microcontroller onto one
210 of the breadboards. Ensure that the microUSB port of the microcontroller faces outwards.
211
212 3.2.    To connect LED modulation, connect the CTL pin of the LED (+) driver to a digital pin of
213 the microcontroller and the LED (-) pin of the LED driver to a GND pin of the microcontroller.
214
215 3.3.    Remove the plastic covers on the back of the breadboards. Press the adhesive backing of
216 the breadboards to the 3D printed part to attach the combined breadboards to the inside of the
217 back portion of the 'LCD_Screen_Holder.stl' printed part.
218
219 3.4.    Secure the liquid crystal display (LCD) screen holder with the assembled breadboards
220 inside to the optical enclosure assembled in section 2 with one-inch-long 4-40 screws.

221

3.5.     To connect the LED power supply, connect the LED (+) pin of the LED driver to the positive wire of the first LED. Connect the negative wire of the first LED to the positive wire of the second LED on the breadboard. Connect the negative wire of the second LED to the LED (-) pin of the LED driver.

NOTE: The order of first or second LED is arbitrary.

3.6.     To connect the LED driver power supply, connect the positive and negative wires of the 10 V power supply to the VIN+ and VIN- pins of the LED driver, respectively. (A barrel jack to two-pin adapter was used.)

3.7.     Connect the sensor test board power supply and data transfer. Only four pins on the sensor test board are used: the SCK, SDA, VDUT, and GND. Take a 4-pin female to male jumper wire and connect those 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.

3.8.     On the breadboard, ensure connections between the following are in place: the 3.3 V pin of the microcontroller and the VDUT pin of both test boards; the GND pin of the microcontroller and the GND pin of both test boards; the analog 4 (A4) pin of the microcontroller and the SDA pin of both test boards; and the analog 5 (A5) pin of the microcontroller and the SCK pin of both test boards.

NOTE: Because $I^2C$ communication is used for the light sensors, the SCK and SDA pins of both sensors can both be routed to the same pins of the microcontroller.

3.9.     Secure the single-board computer onto the LCD Screen holder with four M2.5 screws. Ensure that the HDMI and power adapter ports of the single-board computer face upwards and the single-board computer is centered on the 3D printed part.

3.10.    Connect the touchscreen display to the single-board computer as per the touchscreen instructions, and then connect the HDMI port of the single-board computer to the touchscreen's HDMI port.

**4. Software installation**

4.1.     Install and use the web editor to upload the custom sketch "MiniFluorimeter_2Diode.ino" provided in **Supplemental Coding File 1** onto the microcontroller. Ensure the "ClosedCube OPT3002" library is installed using the Library Manager.

4.2.     Change the variable led_A_pin to the number of the digital pin used in step 3.3 (Electronics and touchscreen assembly section).

4.3.     Adjust the number of milliseconds the LED is turned on, change the value of the variable

265 ExposureTime to acquire fluorescence measurements. To adjust the number of milliseconds
266 between LED exposures by change the value of the variable led_A_Interval.

268 4.4.    Change the variable led_Power to a number between zero and one to adjust the
269 brightness of the LEDs during exposures. Zero gives the maximum amount of brightness and one
270 gives the lowest amount of brightness.

272 4.5.    Turn on the ability to control the display via touchscreen by following the manufacturer's
273 instructions provided with the 3.5-inch display.

275 NOTE: If desired, the 3.5-inch screen can be used as a monitor without touchscreen capabilities,
276 and a keyboard and mouse can be attached to the USB ports of the single-board computer for
277 control of the single-board computer.

279 4.6.    Download the "MiniFluorimeter_2Diode_GUI.py" file from **Supplemental Coding File 2** to
280 a desired location on the single-board computer.

282 4.7.    Ensure a working version of Python is installed on the single-board computer . Python 3.7
283 was used in the provided Python module, but any stable Python version could be used with
284 appropriate changes to the provided script. Install the libraries needed for the Python program
285 onto the single-board computer.

287 4.8.    Change the variable measurement_time to the length of time desired to take
288 measurements. The program ends acquisition and closes after the desired time is elapsed. The
289 GUI also allows to end acquisition through a button on the user interface.

291 4.9.    Change the variable serialPort to the serial address of the connected microcontroller.

293 **5. Recording real-time fluorescence data**

295 5.1.    Turn on the commercial heat block and allow it to reach the desired temperature.

297 5.2.    Power the single-board computer with a standard 5 V power supply provided with most
298 single-board computer purchases. Connect the single-board computer to the microcontroller
299 with a microUSB to USB cable.

301 5.3.    Using the touchscreen, open the provided Python script. Change the measurement_time
302 and serialPort variables to the desired values. Change the variable outputFilepath to the name of
303 the data file the program generates. Ensure the filename ends in '.xlsx'.

305 5.4.    Place two PCR tubes containing the reactions to be monitored into the heat block. Ensure
306 that the placement of the PCR tubes aligns with the optical channels of the fluorimeter once it is
307 placed onto the heat block.

308

309 <mark>5.5.    Place the fluorimeter atop the heat block with the PCR tubes centered between the four</mark>
310 <mark>pegs extruding from each optical channel of the fluorimeter. For optimal measurements, ensure</mark>
311 <mark>that the 3D printed fluorimeter is attached securely into the wells of the heat block.</mark>

313 <mark>5.6.    Securely attach the fluorimeter, plug in the power supply adapter for the LEDs.</mark>

315 <mark>5.7.    Use the touchscreen to start the Python program. A graphical user interface (GUI) appears</mark>
316 <mark>on the LCD screen and measures the real-time fluorescence.</mark>

318 <mark>5.8.    Observe the real-time fluorescence measurements over time for both PCR tubes that are</mark>
319 <mark>shown to the user on the GUI.</mark>

321 <mark>5.9.    After the user-determined experiment time signal, acquisition ceases. View the</mark>
322 <mark>measurements in the output data file saved in the user-defined location. To end the</mark>
323 <mark>measurements early, click the button labeled "Stop Acquisition" on the user interface.</mark>

325 **REPRESENTATIVE RESULTS:**
326 Once assembled, fluorimeter performance can be validated by measuring fluorescence from a
327 dilution series of FITC dye. In **Figure 3A**, measurements of FITC dye at concentrations of 0, 20, 40,
328 60, and 80 pg/µL prepared in 1x PBS on both channels of the first configuration of the fluorimeter
329 are shown. Each sample was measured three times with an LED exposure of 1.5 s at 20 s intervals.
330 Both channels of the fluorimeter show a linear response across the desired range.

332 Clinical applicability of the fluorimeter was further demonstrated by using the system together
333 with a commercially available dry heat block to perform amplification with two isothermal
334 amplification technologies: RPA and RT-LAMP.

336 **Figure 3B** demonstrates the baseline-subtracted time course of fluorescence measured during
337 39 °C amplification of 50 µL of real-time RPA positive and negative control reactions for kit
338 positive control DNA provided in a standard commercial kit and prepared per manufacturer's
339 instructions. RPA reactions, which produce a relatively low level of fluorescence, were measured
340 using the first configuration of the fluorimeter that achieves better suppression of excitation
341 light.

343 **Figure 3C** demonstrates the time course measurement of a custom RT-LAMP assay at 65 °C
344 utilizing the N2, E1, and As1e primer sets described by Zhang et al[10], and Rabe and Cepko[11]. RT-
345 LAMP reactions produce a greater amount of fluorescence and were measured using the second,
346 lower-cost fluorimeter configuration. Oligonucleotides were purchased and resuspended in 2x
347 TE buffer at a 1 mM concentration. Forward inner primer (FIP) and backward inner primer (BIP)
348 oligos were ordered with high performance liquid chromatography purification. Each primer set
349 (N2, E1, and As1e) was combined to make 1000 µL of a 25x mix as follows: 40 µL of FIP, 40 µL of
350 BIP, 5 µL of F3, 5 µL of B3, 10 µL of LF, 10 µL of LB, and 890 µL of 1x TE buffer. To assemble each
351 RT-LAMP reaction, 1 µL of each primer set was added to 0.5 µL of 50x fluorescent dye and 12.5
352 µL of 2x master mix and the reaction volume was brought up to 20 µL of with nuclease-free water

per manufacturer's instructions. The SARS-CoV-2 RNA Control was serially diluted in nuclease-free water to concentrations of 10, 100, or 1,000 copies per µL, and 5 µL were added for a total reaction volume of 25 µL. The no target control (NTC) used in all experiments was nuclease-free water. RT-LAMP reactions were overlayed with 25 µL of molecular biology-grade mineral oil.

RPA and RT-LAMP reactions were assembled in two wells of a 0.2 mL of low-profile 8-tube PCR strip and capped with ultraclear flat caps. Each RPA and RT-LAMP reaction was run in triplicate. In all tests, the mini-fluorimeter successfully quantified the temporal increase of fluorescence levels associated with DNA amplification.

**FIGURE AND TABLE LEGENDS:**
**Figure 1: Optical housing and assembled miniature fluorimeter atop heat block.** (**A**) Diagram of optical housing showing optical components placed in one detection channel. (**B**) Diagram of the first configuration of the miniature fluorimeter following assembly. (**C**) Photograph of the optical housing with optical components placed in one detection channel. (**D**) Photograph of assembled miniature fluorimeter placed on top of a commercially available heat block.

**Figure 2: Assembly and electrical control diagram of the miniature fluorimeter. A-J**) Step-by-step placement of the optical components in the 3D printed optical housing for the first system configuration. (**K**) Electrical diagram of the miniature fluorimeter for both configurations.

**Figure 3: Representative measurements obtained with the miniature fluorimeter**. (**A**) Measured fluorescence vs. FITC dye concentration in both channels shows linear response across the desired dynamic range. (**B**) Real-time fluorescence vs time for isothermal amplification of positive and negative controls of a commercially available kit. Amplification occurs as expected for the positive control. (**C**) Real-time fluorescence vs time for isothermal amplification of 50 copies of SARS-CoV-2 RNA and an NTC sample from a custom RT-LAMP assay. Amplification occurs as expected near the limit of detection of the assay.

**Table 1: Cost comparison of the two configurations of the miniature fluorimeter.**

**Supplemental File 1: System1_Optics_Enclosure_Top.stl**

**Supplemental File 2: System1_Optics_Enclosure_Bottom.stl, and**

**Supplemental File 3: LCD_Screen_Holder.stl**

**Supplemental File 4: System2_Optics_Enclosure_Top.stl**

**Supplemental File 5: System2_Optics_Enclosure_Bottom.stl, and**

**Supplemental File 6: System2_BuildInstructions.pdf**

**Supplemental Coding File 1: MiniFluorimeter_2Diode.ino**

397

**Supplemental Coding File 2: MiniFluorimeter_2Diode_GUI.py**

399

**DISCUSSION:**
Described here is an open-source, low-cost, modular, portable fluorimeter for quantitative fluorescence detection of isothermal amplification reactions. Open-source projects facilitate rapid and inexpensive maintenance with readily available replacement parts and allow users the flexibility to adapt the system to their needs based on modular design. This protocol describes the process of assembling mechanical, optical and electrical components, and validating optical performance. Furthermore, the flexibility of the fluorimeter to monitor two different types of isothermal amplification assays with significantly different temperature, volume, and fluorescence requirements, RPA exo and RT-LAMP, was demonstrated. RPA is performed at 39 °C in 50 µL reactions that utilize a sequence-specific FAM-tagged probe for fluorescence generation, while RT-LAMP is performed at 65 °C in a 25 µL reaction volume and uses an intercalating dye to report the presence of the amplified DNA. Because fluorescence measurements are made through the top of PCR tubes with flat caps, the fluorimeter is able to detect fluorescence from both assay volumes, and heat requirements are only limited by the commercial heat block chosen. Furthermore, the fluorescence intensity produced in RT-LAMP is almost in the order of magnitude greater than that produced in RPA, due to the dye- versus probe-based methods of fluorescence signal generation. However, the dynamic range of the optical sensor chosen can detect and quantify both the signals, and baseline subtraction algorithms account for these differences to produce reliable fluorescence readings.

To facilitate technology dissemination and minimize potential maintenance cost, a modular design that is compatible with heaters that are widely available in different settings was employed. In the current protocol, a common dry block heater was used; the same optical and electrical design can be readily adapted for other commercially available heaters. If another dry block heater is to be used, minimal changes in the 3D housing design will be required. Specifically, the bottom pegs of the optical enclosure STL files must be modified to ensure proper alignment with the wells of other commercial heat blocks. While the enclosures shown in the examples were printed on a relatively low-end 3D printer (see **Table of Materials**), care should be taken to ensure the printer resolution and/or print tolerances are adequate to accommodate the optical components and threaded inserts. In the STL files provided, a tolerance of 0.01–0.02 inches was added on either side of the optical components in the radial and axial directions based on the dimensions specified by the manufacturer. This ensures all optical components fit securely within the print and that the enclosure completely blocks excess light from entering or escaping. To ensure a proper press fit for the threaded inserts, a similar tolerance of 0.01–0.02 inches was subtracted from the manufacturer-provided diameter in the CAD file.

RPA reactions were successfully monitored using the first fluorimeter configuration, while RT-LAMP reactions could be monitored using either configuration. The improved stray light rejection of the first configuration was necessary to monitor the low levels of fluorescence produced by the fluorogenic probe in RPA reactions. In contrast, RT-LAMP utilizes an intercalating dye for signal generation, resulting in a higher fluorescence intensity that is compatible with the lower

dynamic range of the second configuration using photographic filter foils. Users should select the fluorimeter configuration that matches the fluorescence signal generating element—intercalating dye or fluorogenic probe—of their assay.

One limitation of this system is that heating is provided by a commercially available heat block powered through a standard wall outlet. This system could be further developed for use in areas lacking reliable access to electricity by incorporating portable and rechargeable battery packs as shown by other groups[12]. Another limitation is the relatively low throughput of the system, which allows for simultaneous fluorescence measurement of only two samples at a time. Multiple prints of the enclosure can be placed atop the same heat block to increase throughput; however, the light sensor used only has four unique I2C addresses. This restricts the maximum number of samples that can be simultaneously measured to four. A different light sensor with a larger number of unique $I^2C$ addresses is needed to further increase throughput.

**DISCLOSURES:**
The authors declare no conflicts of interest.

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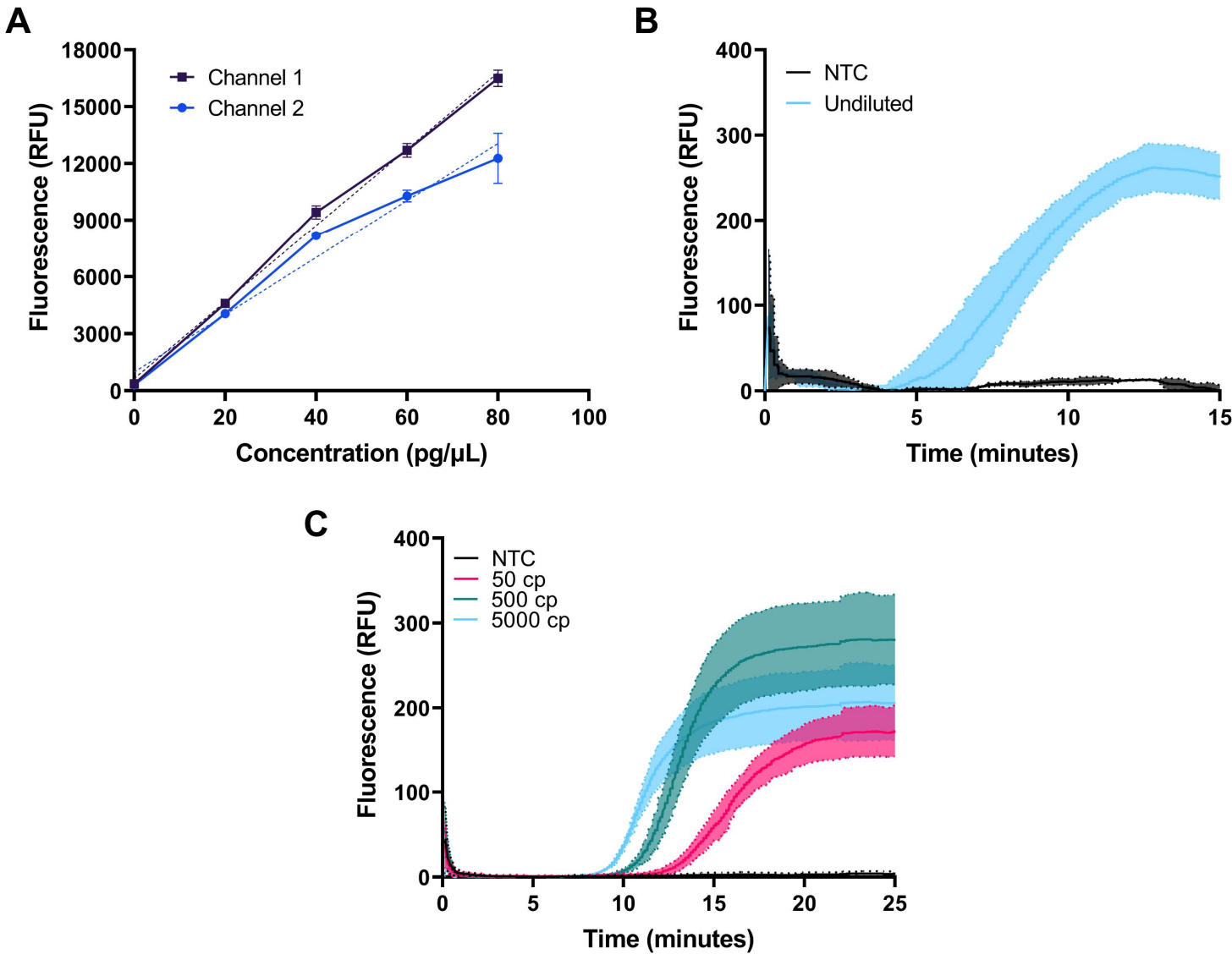492 a Flame or Electricity. *Nature Biomedical Engineering*. **2** (9), 657–665 (2018).

493
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Figure 1 Click here to access/download;Figure;Figure1.pdf



0.5 in

Figure 2                                                    Click here to access/download;Figure;Figure2.pdf ☒

Figure 3

Table 1
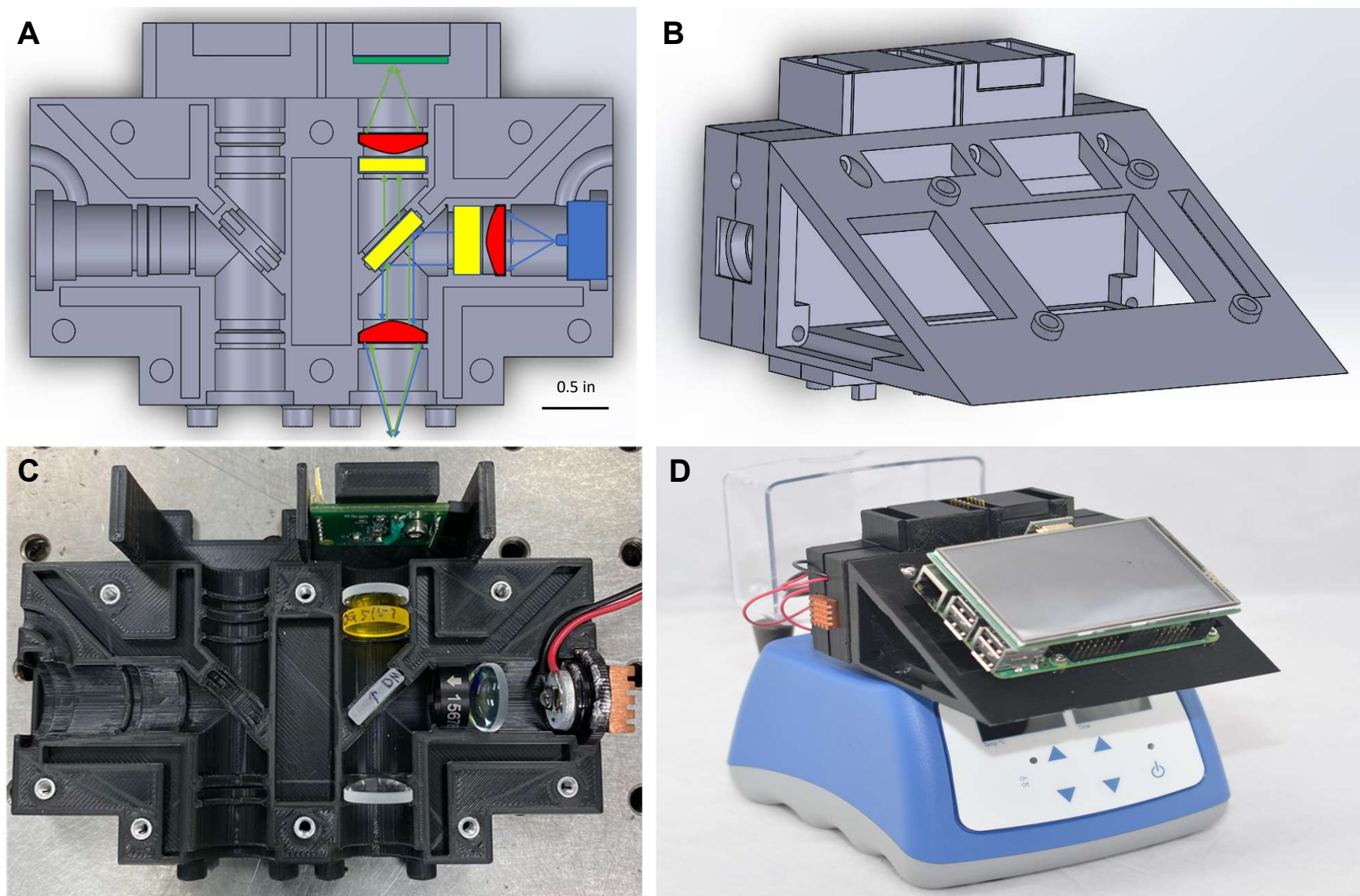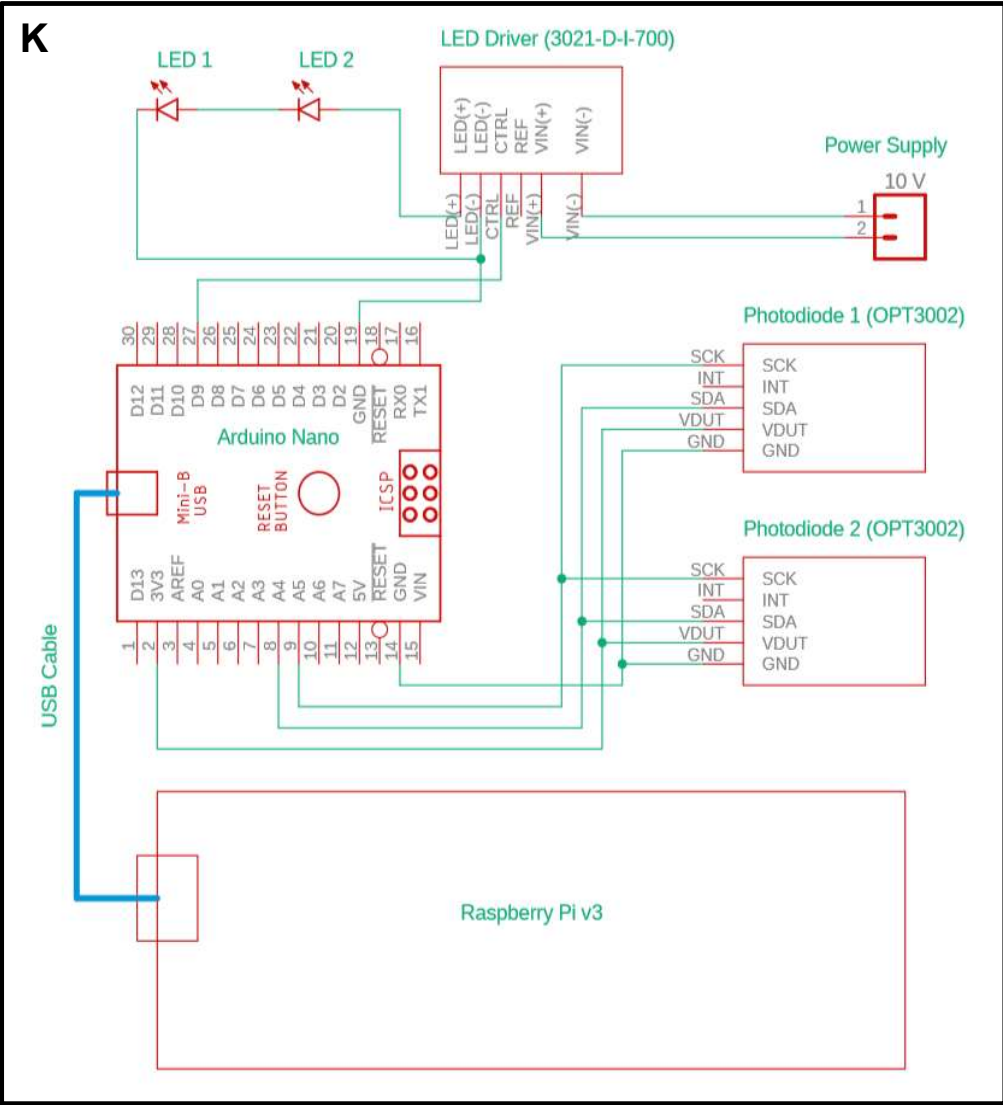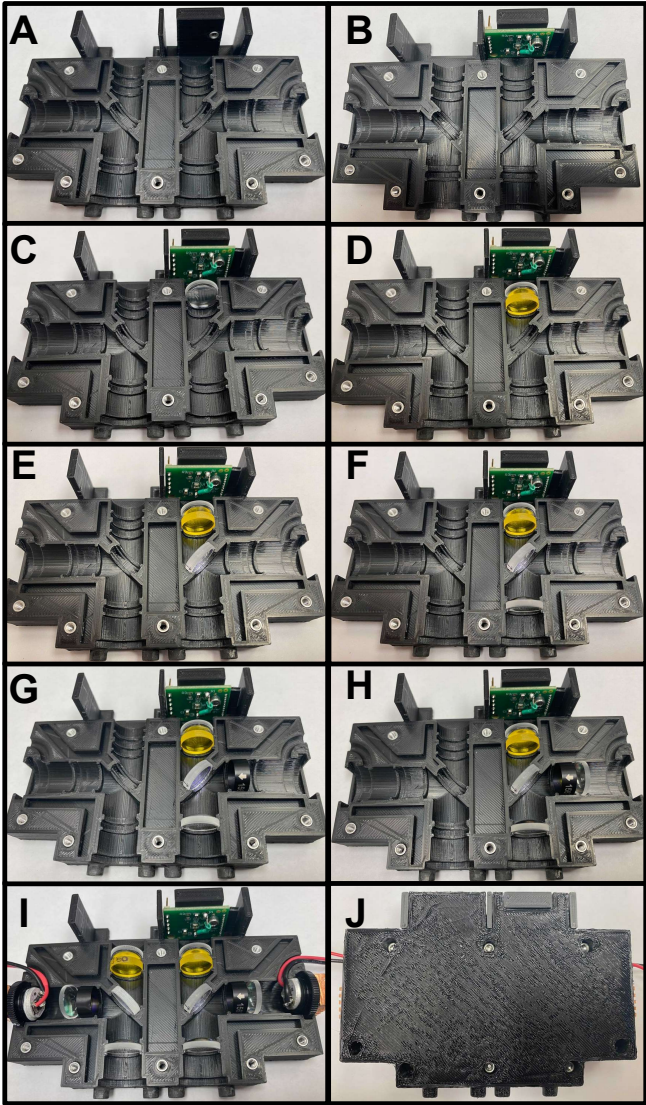
| Item | System 1 | | System 2 | |
|---|---|---|---|---|
| | Quantity | Total Price (USD) | Quantity | Total Price (USD) |
| **Optical Components** | | | | |
| Lenses | 6 | 158.14 | 6 | 158.14 |
| Mirrors | 2 | 244.56 | 2 | 60 |
| Optical Filters | 4 | 200 | 6 | 5 |
| | **Subtotal** | 602.7 | **Subtotal** | 223.14 |
| **Illumination and Detection** | | | | |
| LEDs | 2 | 72.62 | 2 | 72.62 |
| LED Driver | 1 | 11.49 | 1 | 11.49 |
| Photodiode | 2 | 50 | 2 | 50 |
| | **Subtotal** | 134.11 | **Subtotal** | 134.11 |
| **Electronics and Display** | | | | |
| Arduino Nano | 1 | 20.7 | 1 | 20.7 |
| Raspberry Pi | 1 | 35 | 1 | 35 |
| LCD Screen | 1 | 25 | 1 | 25 |
| Mini Breadboard | 1 | 4 | 1 | 4 |
| 10V Power Supply | 1 | 8.6 | 1 | 8.6 |
| | **Subtotal** | 93.3 | **Subtotal** | 93.3 |
| Total Cost (USD) | | 830.11 | | 450.55 |

| Name of Material/Equipment | Company | Catalog Number |
| --- | --- | --- |
| 1/4-inch-long 4-40 threaded insert | McMaster-Carr | 90742A116 |
| 10v power supply | GlobTek, Inc. | WR9HU1800CCP-F(R6B) |
| 15 mm focal length lens | Thorlabs | LA 1074 |
| 1-inch-long 4-40 screws | McMaster-Carr | |
| 20 mm focal length lens | Thorlabs | LA 1540 |
| 2x WarmStart LAMP Master Mix | New England Biolabs, Inc | E1700 |
| 3.5" Touch Screen | Uctronics | BO10601 |
| 3/16-inch-long 4-40 screw | McMaster-Carr | 90128A105 |
| 3/16-inch-long 4-40 threaded insert | McMaster-Carr | 90742A115 |
| 3/8-inch-long 4-40 screws | McMaster-Carr | 90128A108 |
| 3D printer filament | 3D Universe | UMNFC-PC285-BLACK |
| 3D printer used | Ultimaker | Ultimaker 2+ |
| 8-tube PCR strips | BioRad | #TLS0801 |
| Advanced Mini Dry Block Heater | VWR International | 10153-320 |
| barrel jack to two-pin adapter | SparkFun Electronics | 1568-1238-ND |
| Blue Excitation Filter Foil | LEE | LE071S |
| Blue LED - 460 nm | Mouser | LZ1-30DB00-0100 |
| Dichroic Mirror | Thorlabs | DMLP505T |
| Emission Filter | Edmunds Optics | OG-515 |
| Excitation Filter | Omega Filters | 490AESP |
| LED Driver | LEDdynamics | 3021-D-I-700 |
| M2.5 Hex Shaped insert | McMaster-Carr | 91292A009 |
| Microcontroller | Arduino | Nano |
| Mini Breadboard | Adafruit | 65 |
| Molecular biology-grade mineral oil | Sigma Aldrich | 69794 |
| OPT3002EVM - Light-to-Digital Sensor | Texas Instruments | OPT3002EVM: |
| Purchased oligonucleotides | Integrated DNA Technologies | |
| RPA kit positive control DNA | TwistDx Limited | CONTROL01DNAE |

| SARS-CoV-2 RNA Control | Twist Biosciences | MN908947.3 |
| Single board computer | Raspberry Pi | Raspberry Pi 3 |
| TwistAmp RPA exo kit | TwistDx Limited | TAEXO02KIT |
| Ultraclear flat caps | BioRad | #TCS0803 |
| Yellow Emmission Filter Foil | LEE | LE767S |

**Comments/Description**

Used to secure the two sides of the 3D printed optical enclosure together.

AC/DC Wall Mount Adapter 10V 18W

Two total are used for the fluorimeter. This lens is used to focus the LED illumination.

Four total are used for the fluorimeter.

Master mix was used to create the LAMP reactions shown in Figure 3C

Used to secure the OPT3002 test board onto the 3D printed enclosure

Used to secure the two sides of the 3D printed optical enclosure together.

Black or another dark color preferred

The following heat blocks are acceptable substitutes without the need for redesigning the optical assembly: 949VWMNLUS, 949VWMHLUS, and 949VWMHLEU

Selected for use with FITC - other fluorescent dyes may require different filters.

Selected for use with FITC - other fluorescent dyes may require different parts

Selected for use with FITC - other fluorescent dyes may require different parts

Selected for use with FITC - other fluorescent dyes may require different parts. The arrow on the part points away from the illumination source.

Selected for use with FITC - other fluorescent dyes may require different parts

Used to secure the Raspberry Pi to the 3D printed LCD Screen Holder

Light-to-digital sensor used. Consists of two PCBs: a SM-USB_DIG board and the OPT3002 test board; only the OPT3002 test board is needed for this device.

Selected for use with FITC - other fluorescent dyes may require different parts

# Author Response

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

**<u>Editorial comments:</u>**

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. Please define all abbreviations at first use.

We carefully reviewed the manuscript to correct spelling and grammar issues.  We defined all abbreviations at first use.

2. Please provide an email address for each author.

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3. 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 and Reagents. For example: 949VWMNLEU Advanced 100 Mini Dry Block Heater (VWR #10153-320), 949VWMNLUS,102 949VWMHLUS, and 949VWMHLEU; Texas Instruments; OPT3002; LZ1-30DB00-0100 LED Engins (Mouser); Adafruit; TwistDx Limited; TwistAmp RPA exo kit; Integrated DNA Technologies (IDT), Coralville IA; WarmStart LAMP Master Mix (New England BioLabs Inc, E1700); Twist Biosciences, MN908947.3; BioRad, #XYZ; VWR Advanced Dry Block heater etc

We replaced commercial language with generic terms throughout the manuscript.  All commercial products are now referenced in the Table of Materials and Reagents.

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

We removed personal pronouns from the text of the revised manuscript.

5. Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. 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. Please add more specific details (e.g., button clicks for software actions, numerical values for settings, etc) to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

We expanded the protocol sections to more fully address how each step is performed. We also expanded the "Software Installation" and "Recording real-time fluorescence data" sections to provide more specific detail on how to perform the protocol.

6. 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 and one-inch margins on all the side. Please include a one line space between each protocol step and then highlight up to 3 pages of protocol text for inclusion in the protocol section of the video.

We formatted the manuscript as directed.

7. Please remove the embedded Table of Materials from the manuscript. All tables should be uploaded separately to your Editorial Manager account in the form of an .xls or .xlsx file.

The embedded Table of Materials has been removed from the manuscript and attached to the submission as a .xlsx file.

8. As we are a methods journal, please revise the Discussion to 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

We expanded the Discussion section to explain critical and troubleshooting steps, including:

1) how to adapt the 3D printed enclosure to work with a variety of commercial heat blocks;
2) how and why to ensure the system is tightly sealed; and
3) how to further reduce the cost of the fluorimeter based on the type of isothermal assay being performed.

Finally, we added a section discussing the current limitations of our technique.

9. 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. Please include volume and issue numbers for all references, and do not abbreviate journal names.

We reformatted the references using the requested format.

**Reviewer #1:**

Manuscript Summary:

A nice piece of work. Clearly presented and reproducible build instruction are included. Needs a little more data to prove the system works.

Major Concerns:

Only one reaction for RPA and one reaction for LAMP is presented. This is a nice system and additional evidence to show efficacy and reproducible would make it valuable to the reader .To access the validity, utility and reproducibility of the system described, more representative results need to be presented. At a minimum 3 repeats of the positive and negative controls should be presented, ideally a serial dilution of the template would offer the ability to assess the performance of the system with different template starting quantities.

We thank the reviewer for their feedback, which helped us improve our manuscript. We added results to show data from a clinically relevant range of input copy numbers for RT-LAMP in triplicate. For RPA, we added results to show the positive and negative controls in triplicate; it was not possible to provide data with a range of input copy numbers due to the low concentration at which the RPA exo kit's positive control DNA is supplied.

In addition, we further reduced the cost of the system (from $830 to $450) by developing a configuration that uses lower-cost optical filters. We show that the lower-cost system can be used to monitor fluorescence generated in LAMP reactions, which use an intercalating dye to generate signal.  RPA reactions, which rely on a fluorogenic probe, are best monitored using the original system.

Minor Concerns:

Figure 3a lists an absolute fluorescence intensity on the y axis as an irradiance quantity but I can not see what this irradiance quantity refers to. Is this the irradiance on the sensor surface? Can you please clarify where these absolute measures are made?

Our initial submission referred to the fluorescence intensity on the sensor surface (which is calculated using a conversion factor supplied by the manufacturer).  For generality, we revised the manuscript to provide fluorescence in relative fluorescence units (RFU).

**Reviewer #2:**

Manuscript Summary:

The paper describes design and assembly of a modular and low-cost fluorimeter for isothermal diagnostics. The paper demonstrates clinical applicability of the method by performing real-time nucleic acid detection with two different isothermal amplification technologies. This methods are needed for simple diagnostics.

Minor Concerns:

Why fluorescence of the positive control, shown on Fig. 3B, shows significantly lower fluorescence level than 50 copies (Fig. 3C)?

The difference in fluorescence intensity arises because RPA reactions (Fig 3B of initial submission) use a fluorogenic probe and produce less fluorescence than RT-LAMP reactions (Figure 3C) which utilize an intercalating fluorescent dye.

Fig 3C shows signal amplification from 50 copies. Would be useful to demonstrate signal amplification from other concentrations also, i.e., 100, 500 copies.

We thank the reviewer for this feedback, which helped us improve our manuscript. As described above, we added data (measured in triplicate) describing results across a clinically relevant range of copy numbers for RT-LAMP.

**Reviewer #3:**
Manuscript Summary:
This is a detailed description of home made real-time PCR system made by 3d printing and off the shelf components.

Major Concerns:
none

<span style="color:red">We thank the reviewer for their feedback.</span>

October 2nd, 2020

Editor, *Journal of Visualized Experiments*
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Phone: 617-945-9051
Email: submissions@jove.com

Dear Editor,

We are pleased to submit our manuscript titled "Open-Source Miniature Fluorimeter to Monitor Real-Time Isothermal Nucleic Acid Amplification Reactions in Resource-Limited Settings" for consideration to be published in *Journal of Visualized Experiments*. This manuscript provides detailed instructions describing how to build an open-source, modular fluorimeter that is compatible with many low-cost heaters to perform real-time, quantitative isothermal nucleic acid amplification. We believe many of your readers will find the design of our system insightful, especially researchers in the field of point of care diagnostics. We would like to request accelerated review due to the role the described technology could play in COVID-19 response across the world. We are aware that video production in affected areas is postponed and can film the video ourselves. Should the article be of interest, we would welcome a discussion of the best way to proceed with filming.

Please let me know if you require any additional information. We appreciate your consideration of our manuscript.

Sincerely,

Rebecca Richards-Kortum, PhD
Malcolm Gillis University Professor
Department of Bioengineering
Rice University
Houston, Texas, USA
Phone: 713-348-3823
Email: rkortum@rice.edu

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| Title: | Malcolm Gillis University Professor |

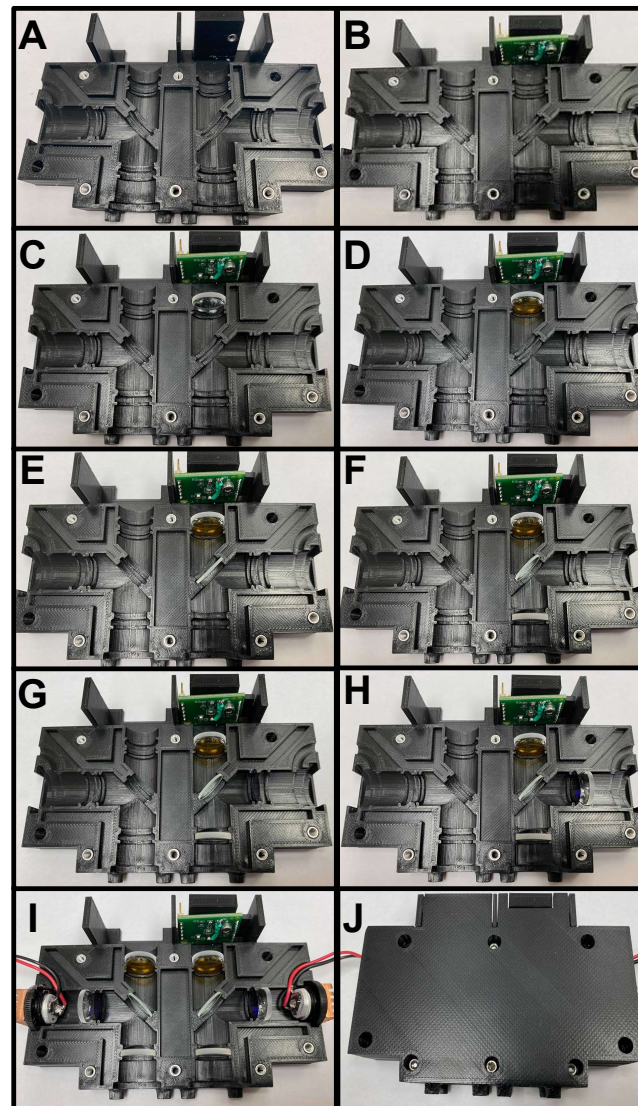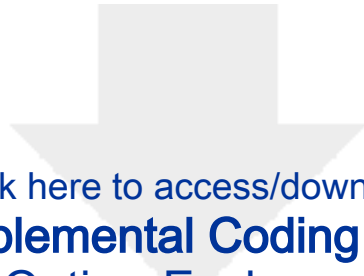| Signature: | *Rebecca R...* | Date: | 10/2/2020 |
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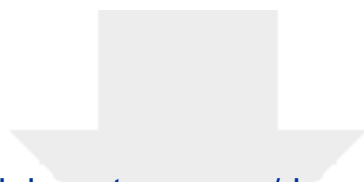
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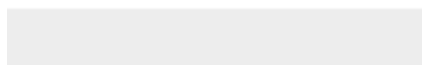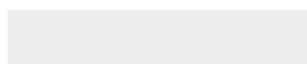System1_Optics_Enclosure_Bottom.STL

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LCD_Screen_Holder.STL

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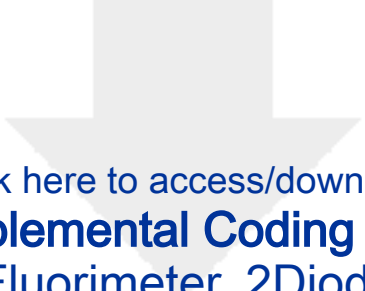System2_Optics_Enclosure_Top.STL

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