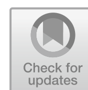
Development of a Low-Cost and Portable Real-Time PCR Machine for Developing Countries



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Abstract Real-time Polymerase Chain Reaction (realtime PCR) is an important medical diagnostic technique in which target DNA is amplified and the amplification process is monitored in realtime. However, most realtime PCR systems are expensive, especially for laboratories in developing countries. To increase accessibility to the realtime PCR technique, we developed a low-cost and portable real-time PCR system. The system was composed of two main parts: a thermocycler and an optical system. The thermocycler's core was a Peltier and a heatsink fan, in tandem with an electronic circuit to control the heating process's temperature. A custom-made fluorescence optical setup was built using a blue LED chip (450 nm), an excitation filter, a dichroic mirror, an emission filter, and a CMOS camera. The results were displayed on a screen connected to a Raspberry Pi module. The thermocycler worked stably and accurately under the control of a PID controller. The system's performance was tested using samples of rice phospholipase D (PLD) gene and maize Bt11 gene. The results showed that, at the end of the amplification process, fluorescence signals from positive samples were significantly higher than from negative samples. Nucleic acid amplification products were verified using gel electrophoresis. With the cost of ~$320 USD, the system could be a suitable candidate for realtime PCR tests in laboratories in rural and remote areas of developing countries.

Keywords Polymerase chain reaction (PCR) · Real-time PCR · Molecular diagnosis · Disease diagnosis

1 Introduction

Polymerase Chain Reaction (PCR) was developed in the 1980s by Kary Mullis, whose pioneering work had awarded him the Nobel Prize in Chemistry in 1994. *Science* magazine said in 1989 that the PCR assay was "revolutionizing the approaches

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researchers are taking to many problems in biology." PCR is an in vitro method that amplifies specific DNA sequences using DNA polymerase's ability to synthesize a new strand of DNA complementary to a given template strand. Due to its feature to add a nucleotide onto a preexisting 3′-OH group, DNA polymerase demands oligonucleotide primers to which it can add the first nucleotide. This condition makes it possible to flank the region of interest in the target DNA. PCR is one of the most popular and robust assays in diagnostic labs to amplify DNA fragments and detect viruses like Ebola [1] and Zika [2]. However, conventional PCR assay requires post-PCR analysis [3], such as gel electrophoresis and image analysis to detect target amplicons' presence and quantity. The post-PCR analysis is labour-intensive and not suitable for high-throughput screening.

Realtime PCR, which is a modification of the PCR technique with the introduction of fluorescence reading during the amplification process, has overcome the aforementioned limits. The ability to monitor the amplification process in real-time makes real-time PCR superior to its predecessor. During the real-time PCR process, the amplification of target sequences generates an increase of fluorescent light directly proportional to the number of PCR amplicons yielded after each PCR cycle. This technique is the gold standard in detecting many different diseases and is very important, especially when the COVID-19 pandemic is threatening lives all around the world.

Nevertheless, in developing countries, real-time PCR is usually only available in laboratories in big cities due to its high cost. Currently, as the COVID-19 is spreading quickly with over 1.7 million infected cases and over 100,000 deaths (as of April 12 2020) [4], there is an urgent need for a low-cost real-time PCR system for laboratories in rural and remote areas of developing countries [5].

In an effort to address this need, we designed an affordable realtime PCR system made of a 3D-printed case and off-the-shelf electronics that provides real-time detection of PCR products. Our realtime PCR system consists of three main parts: a thermal cycler system to regulate temperature, an optical setup to both excite and capture fluorescence signal, and a graphical user interface for system control and result display. The whole size of the device is approximately 350 mm $\times$ 170 mm $\times$ 265 mm. We characterized our system by carrying out real-time PCR reactions. The results showed that the system successfully amplified target DNA and detected fluorescence signal, as verified by gel-electrophoresis results and the acquired fluorescence images.

2 Related Works

Regarding the development of low-cost and portable real-time PCR instrument, several research groups have achieved great advances in making fully functional low-cost real-time PCR systems [6–8]. Many of the groups depended on Peltier as the core of their thermocyclers for heating and cooling and used thermistor to measure temperature [6–11]. This proved to be an effective and economical way to perform the thermocycling process. Regarding optical set-up for fluorescence detection, different fluorescence excitation methods have been deployed, such as putting

LEDs on the sides of the test tubes for direct excitation [6–8, 11–13] or using LEDs array in combination with dichroic mirror for excitation from the top of the test tubes. Each method has its advantages and disadvantages. Despite the differences, the results of these methods are quite encouraging [6–13].

3 Materials and Methods

Our real-time PCR system consists of three main parts, including: (1) a thermocycler system; (2) an optical system; (3) an electronic system. Figure 1 shows the block diagram of the electronic system. At the heart of the electronic system is an MCU board that control the temperature of the Peltier-based thermocycler using PID algorithm. The temperature was monitored by a resistance thermometer PT100 (temperature range from −243 to 600 °C) connected to the MCU board. Sample solutions were excited by a LED light source, and the generated fluorescence could be detected in realtime by a camera. The system was powered by a 12 V power supply.

3.1 Thermocycler

Figure 2 shows 3D design of the thermocycler. The basis of the thermocycler is a 12 V, 150 W TEC1-12715 Peltier module and a computer heat sink cooler. One side of the Peltier was placed directly on the heatsink with fan blower to disperse

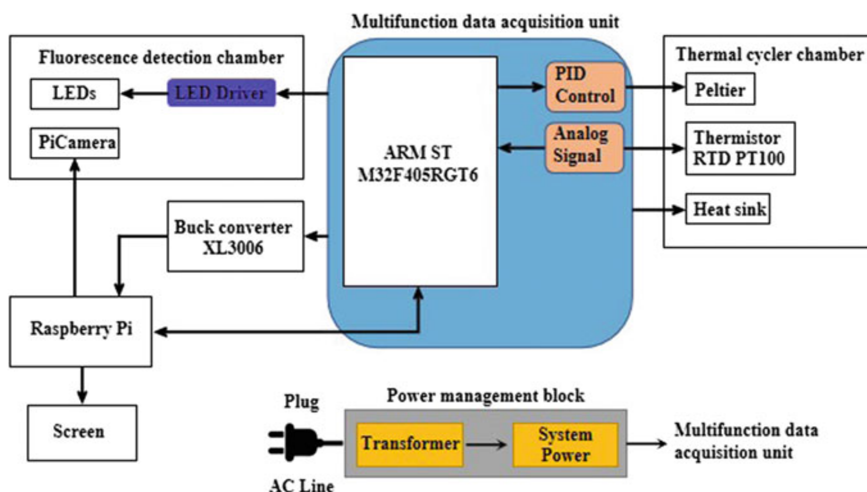

Fig. 1 Block diagram of the electronic system

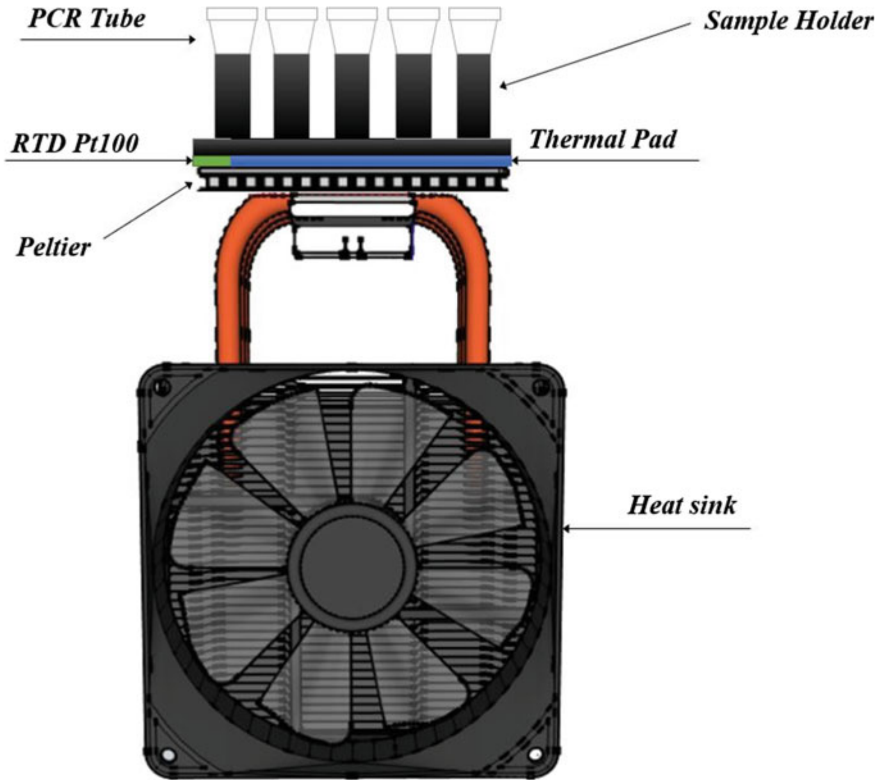

Fig. 2 The thermocycler of the system

the temperature of the heatsink. The heat block, which was salvaged from an old benchtop PCR instrument, was placed on the Peltier's other side. To evenly transfer heat from the Peltier module to the heat block, we put a piece of thermal pad between the Peltier module and the heat block. We also used thermal paste to maximize the thermal transmission effect. The temperature was recorded in real-time by a RTD Pt100 sensor.

The thermal cycler started with a pre-PCR process of 40 °C for 900 s. Then, the PCR reaction was performed with two steps: 95 °C for 20 s and 60 °C for 60 s; the process completed after 40 cycles of heating and cooling.

3.2 Optical System

Aiming to provide a uniform and intensive illumination over the field of view, a 7B10C COB blue LED with 470 nm center wavelength and power capacity up to 30 W was used for fluorescence excitation. Despite the fact that Xeon light source

is chosen by many commercial PCR systems [14–16], we believe that LED is more compact, energy efficient, and more cost-effective for our purpose of building a low-cost device for developing countries. Moreover, the heat generated from Xeon lamp would require an additional cooling system that would increase the whole system's cost.

However, COB LED generates a divergent light beam. Therefore, to focus the light, a cone-shaped beam-restricting tube (Figs. 3 and 4) was designed as a simple light collimator to focus the light and enhance the uniformity of light illumination. The inner surface of the tube was painted with silver paint to increase reflectivity of the surface.

The use of the collimator significantly improved the uniformity of the illumination area created by the COB LED. Intensity maps of the illuminated area with and without the collimator were captured by a Samsung Galaxy S8 smartphone camera and plotted using Matlab. The light source was placed 120 mm away from the projected surface.

Figure 5 shows that, with the collimator, uniformity of intensity and magnitude was significantly improved.

Figure 6 shows the design of the optical system. Light from the blue COB LED went through an 480 nm excitation filter (bandwidth 30 nm), a collimator as described above, and reflected off a 500 nm high-pass dichroic mirror before exciting the samples. Emitted fluorescence with a wavelength over 500 nm will pass through the dichroic mirror, an 525 nm emission filter (bandwidth 15 nm) and captured by a CMOS camera. The emission filter's role is to suppress any residual blue excitation

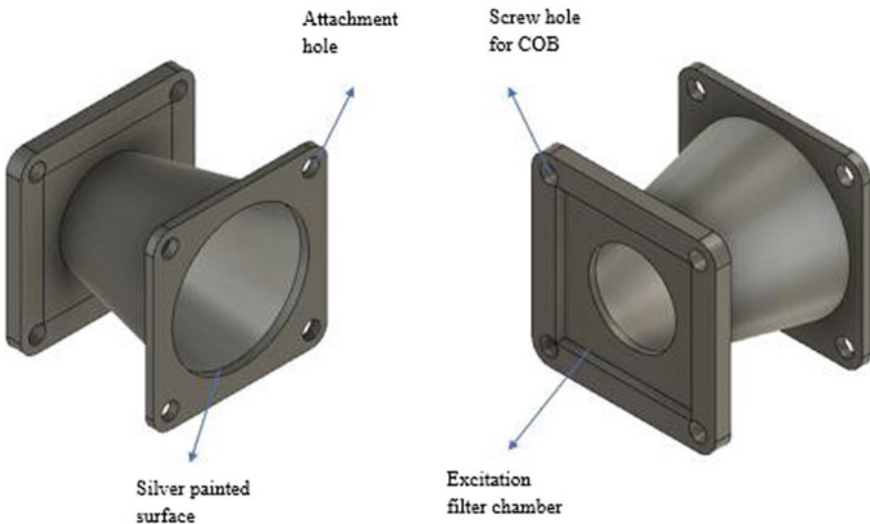

Fig. 3 The 3D model of the collimator viewed from two different angles. The design includes a cavity for excitation filter placement and holes for COB LED attachment. The inner surface of the cone is painted silver, enhancing light reflectivity

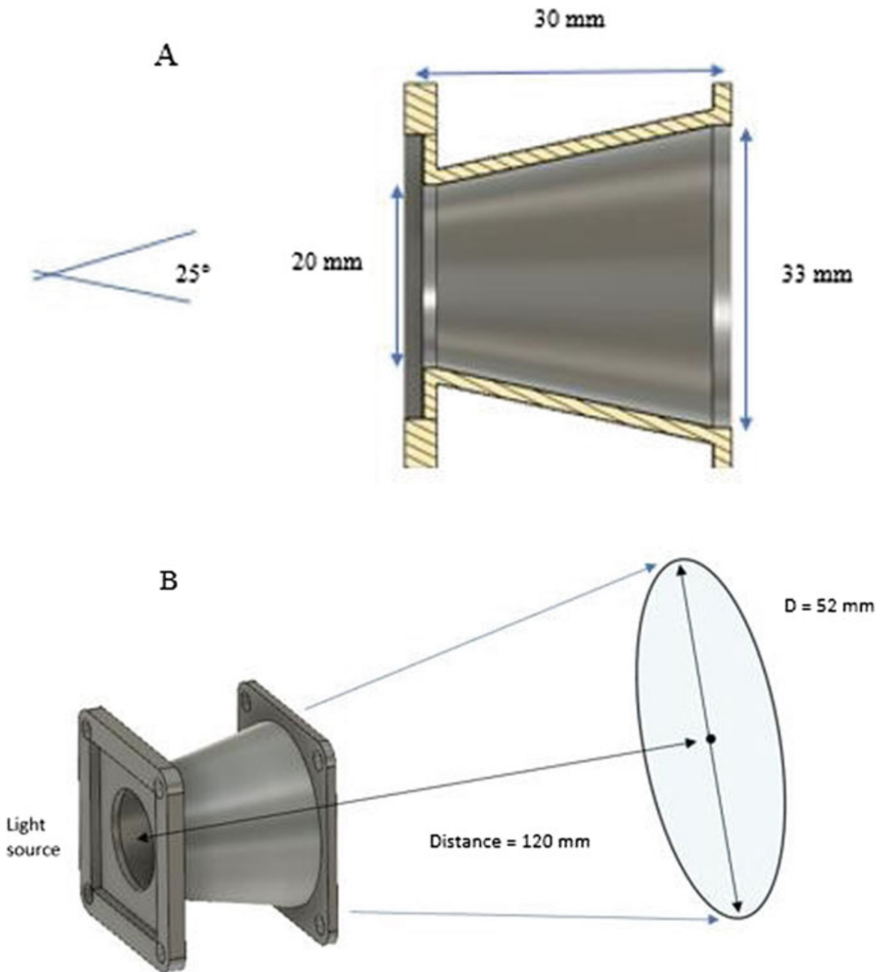

Fig. 4 **a** Cross section of cone-shaped collimator. The cone angle is 25°, the light aperture diameter is 20 mm and the output diameter is 33 mm. **b** The distance from the light source to sample is 120 mm. From the calculation, the circular illumination area has a diameter of 52 mm

light and allow fluorescence emission light to go through and to be collected by the CMOS camera.

3.3 Heat Lid

A heat lid is an important feature of a PCR instrument that prevents samples from condensing inside PCR test tubes. During the denaturation phase of a PCR cycle, the

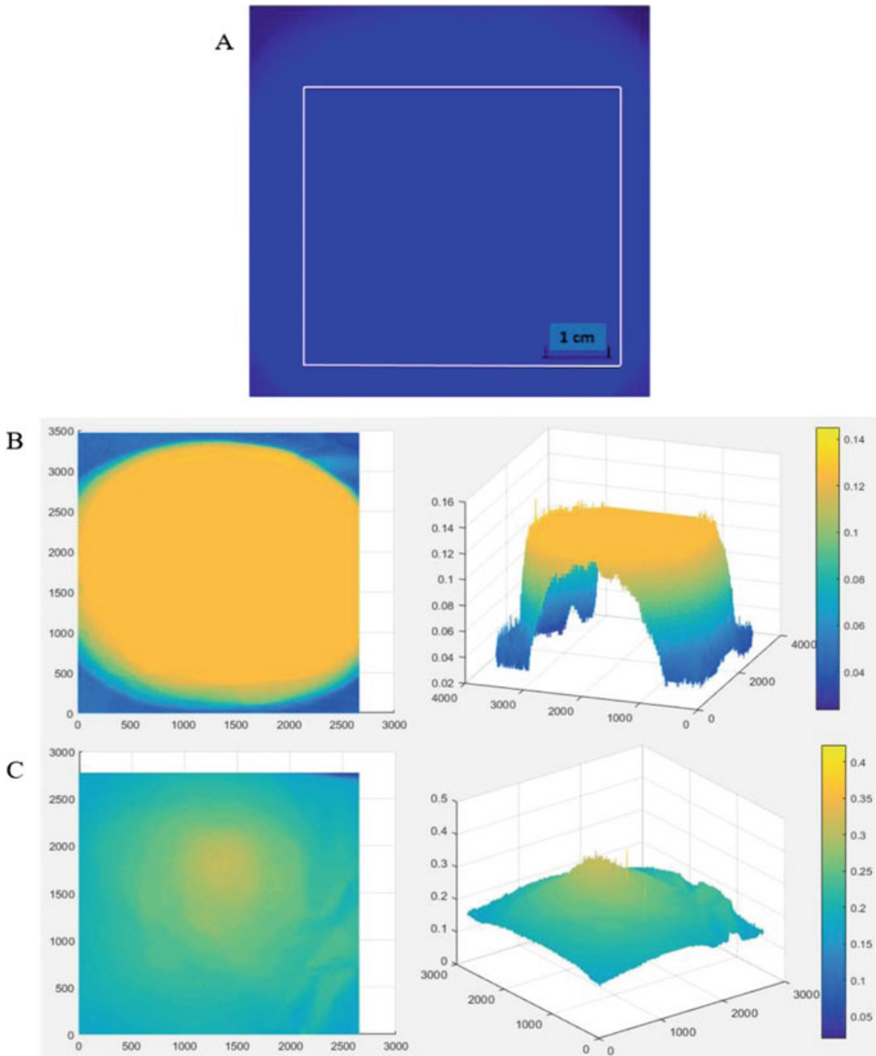

Fig. 5 **a** Area of illumination while attaching the collimator captured by Samsung Galaxy S8 smartphone camera. The white box represents the size of PCR heating block. **b** Total intensity 2D and 3D maps of the illumination area with collimator installed. **c** Total intensity 2D and 3D maps of the illumination area without attaching the collimator. The image is showed in the *xy* plane, the pixel intensity is demonstrated within the *xyz* 3D grid coordinates. The color bars demonstrate the pixel intensity of images. The intensity of the pixels represents the luminous intensity level of the lighting area

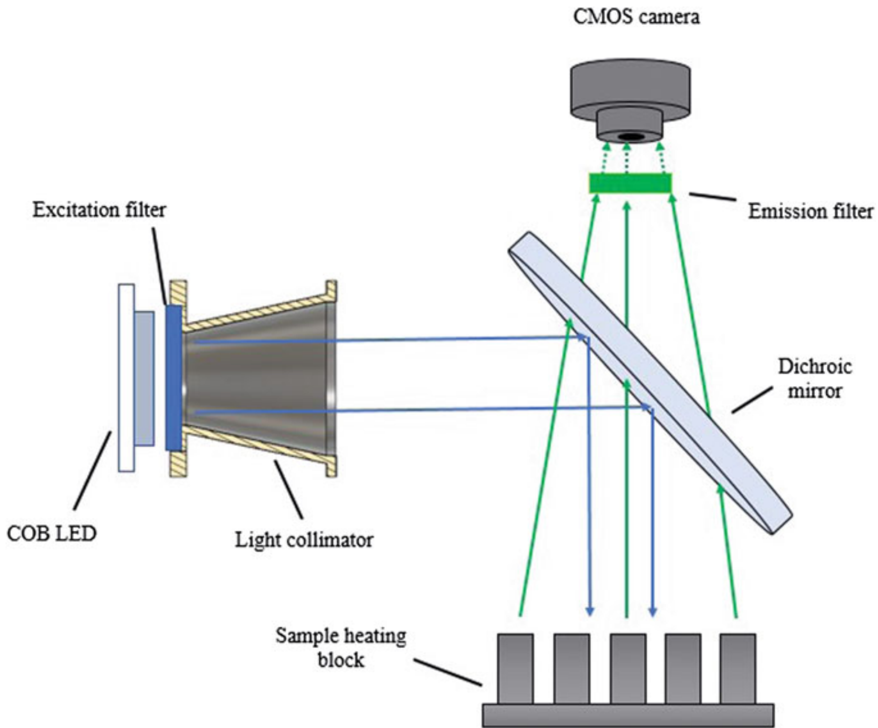

Fig. 6 The optical system of the fluorescence detection system

temperature reaches 95 °C and would cause evaporation and loss of sample, thus after about 25 cycles, there is nothing left on the bottom of the test tubes if no mineral oil is used. Our system has a heated lid to solve this problem. Furthermore, the heated lid allows samples to be heated more efficiently, which reduces the formation of nonspecific annealing and primer dimers.

In this study, two methods of heat lid design were considered. First was to use a transparent Indium Tin Oxide (ITO) coated glass as a heat generator. An ITO heater glass often operates between 80 and 130 °C, it allows 80% of light to transmit through. As a result, ITO heater provides a transparent heat lid that allows light to pass through. This method simplifies the design of the heat lid. However, during our experiments, ITO glass has shown its lack of stability: the temperature generated was not always stable and the soldering process of electric cable to ITO glass was arduous.

Furthermore, it was fragile when being pressed onto test tubes' caps.

Second method was to use an aluminium heater with holes that allow light to transmit through. Figure 7 shows the design of our heat lid. The main component is a Positive Temperature Coefficient (PTC) heater. PTC heater has high heating efficiency, low electricity consumption, cost-effectiveness, and self-regulating ability.

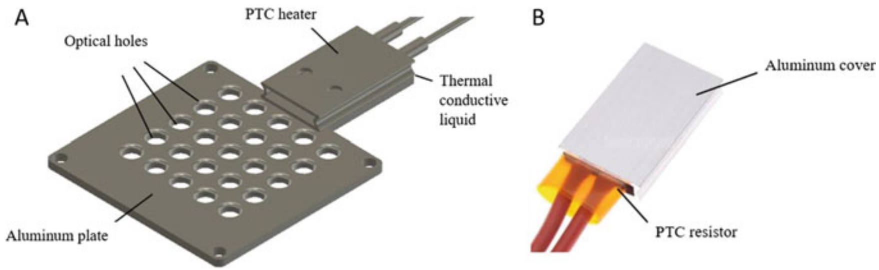

Fig. 7 **a** Heat lid design. An aluminum plate was screwed up with a PTC heater, with a thermal conductive layer in between. **b** PTC heater. PTC resistor is placed inside an aluminum cover, generating heat as electric current passes through

Temperature stability is a crucial reason that we chose PTC heater as the heat generator for our heat lid design. PTC heater was attached to an aluminum plate. Thermal conductive paste was applied between the two components in order to increase heat conductivity.

Heat distribution was not uniform (distant locations have a lower temperature) due to heat dissipation, but the average temperature was stable around 110 °C, which was enough to prevent condensation in test tubes.

4 Results and Discussion

4.1 Fluorescence Acquisition System

Our goal in this project was to develop a low cost and portable realtime PCR instrument that could be easily deployed in low-resource settings to assist the fight against infectious pandemics like Ebola [1], Zika [2] and COVID19 [17]. Our device is powered by an external power supply; however, a portable battery bank can be used as an alternative. Several research groups have reported portable real-time PCR instruments; yet to reduce the size, they relied on costly micro fabrication techniques to produce the heat blocks [7, 11] of the thermocycler. In contrast, our thermocycler leveraged two simple and low-cost components, a peltier module (US$4.3) and a computer heat sink ($30) to heat and cool down the heat block. Moreover, the heat block and the optical system was covered with a 3D-printed enclosure to prevent interference from the outside environment.

For detection of fluorescence signal, we utilized a COB LED ($2) and a CMOS camera ($30) along with an emission filter ($45), an excitation filter ($45) and a dichroic mirror ($60) to filter lights. In terms of controlling the device, an MCU board and a single-board computer, the Raspberry Pi, were used. Our instrument's weight is 3 kg, which can be easily transported and held by an adult. The complete

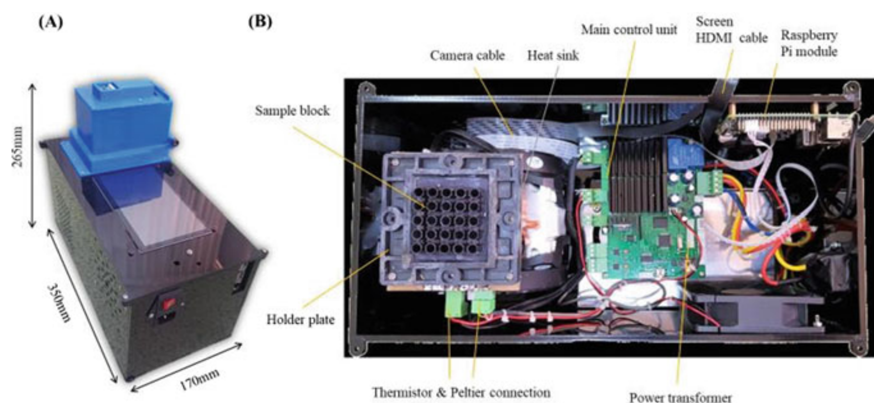

Fig. 8 **a** Our realtime PCR system looks from outside. **b** The internal structure of the device

system is shown in Fig. 8. In its current form, the total cost of the device is $320, which is much cheaper than commercial benchtop real-time PCR (>$4500) [18].

4.2 Temperature Control

To assess performance of the thermocycler and the PID controller, we implemented a PCR protocol that begins with a 15-min hot start at 95 °C, followed by 40 cycles of denaturing and annealing/extension stage at 95 °C and 60 °C, respectively.

Heating and cooling rate of the system could reach 1.4 °C/s (Fig. 9). A 40-cycle PCR reaction is finished in 100 min with 130 s for each cycle to be executed. Besides, the thermal system witnessed a few seconds of temperature overshoot in the first two minutes of the hot start stage. Then the control algorithm was able to cancel the temperature overshoot. To improve temperature ramp rate, Peltier may be replaced with a better quality one because the one that we were using has stated power of 150 W according to its datasheet. However, when it ran at full power, the Peltier was overheated and broken in several trials. So we ran the Peltier at only 120 W power (12 V and 10 A).

To analyze the accuracy of temperature control of the device, three PCR test tubes filled with 25 μL of distilled water were placed at random positions in the heat block during thermal cycling. The temperature of water inside the PCR tubes were measured using a thermocouple connected to a digital multimeter (True RMS Pro's kit MT-1707). Results showed that the system achieved good accuracy in controlling temperature of the solutions in the PCR test tubes (Fig. 10). As for the denaturation step in which set temperature was 95 °C, the actual temperature was 94 °C on average. As for the the annealing/extension step in which set temperature was 60 °C, the actual temperature was 59.8 °C on average.

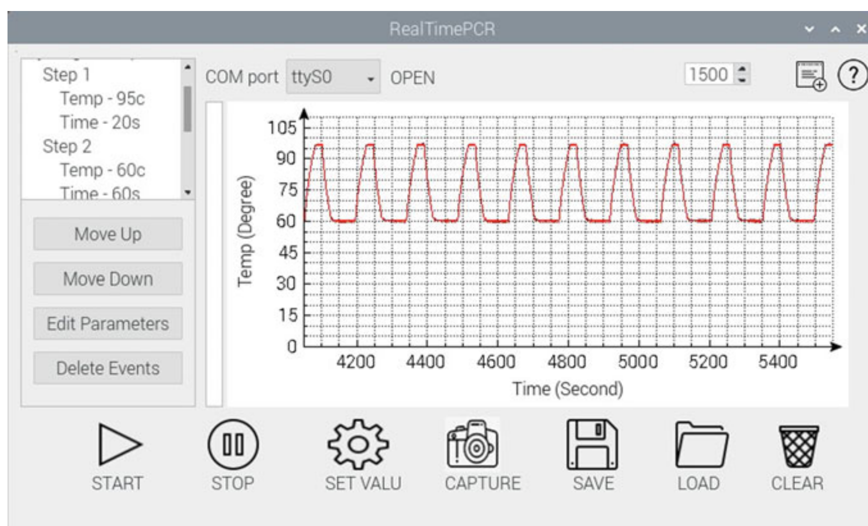

Fig. 9 Shows the thermal graph of the heat block as measured by an RTD Pt100

In terms of temperature ramp rate, our custom-built thermocycler has lower temperature ramp rate in comparison to commercial devices as shown in Table 1. However, our system has a much lower cost. The system could be easily adapted to perform isothermal assays which are gaining popularity recently [2, 10]. For example, Abbott has just released a 5-min diagnostic device for COVID-19 testing, called the Abbott ID Now™. The device uses an isothermal assay operating at around 56 °C for 5 min to give a positive result and 8 more minutes for negative one [17].

4.3 Optical Setup

The optical setup successfully detected fluorescent signal (Fig. 11). Figure 11a shows the captured image of 8 PCR samples with 4 positive samples on the left and 4 negative samples on the right. The tubes were put horizontally, and DNA samples stayed at the bottom of each tube. Figure 11b shows another test with 1 negative sample on the left and 1 positive sample on the right. This time the test tubes were put into the heat block. The images show clear difference between fluorescence of positive sample and fluorescence of negative sample. Gel electrophoresis results confirmed the successful amplification of target DNA in positive samples. It can be concluded that the optical setup functioned effectively.

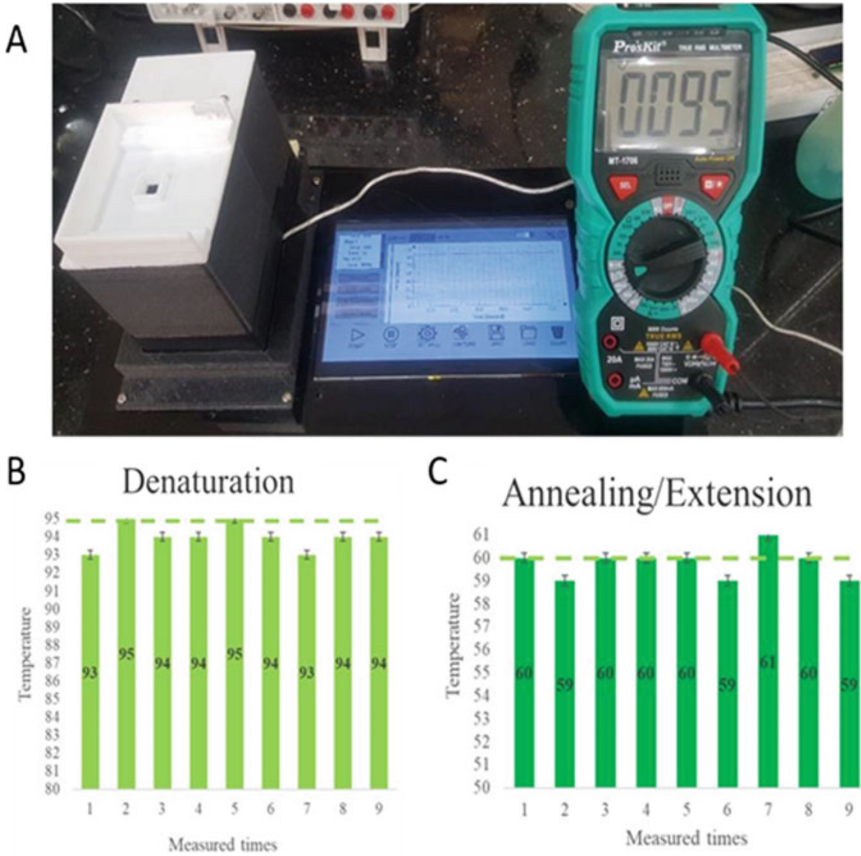

Fig. 10 **a** A Pro'sKit digital multimeter connected to a thermocouple was used for verifying temperature of solutions inside PCR test tubes; temperature of the pre-denaturation step was verified to be at 95 °C. Bar chart illustrates the set values and the actual temperatures of the denaturation stage **b,** and the annealing/extension stage **c**

4.4 Real-Time PCR

To determine the system's practical value, AccuPid Rice PLD Detection Kit [19] and AccuPid Bt11 Maize Detection Kit [20] were used. Positive and negative control samples were taken from the kits and run under two devices: our realtime PCR system and a commercial PCR Eppendorf MasterCycler Nexus Gradient with the same protocol: 15 min at 95 °C, followed by 40 cycles of 95 °C for denaturation and 60 °C for annealing/extension. In our system, after every 5 cycles, fluorescence images were captured. Each image was analyzed with ImageJ to find the average fluorescence intensity of the negative samples and the positive samples. Figure 12 shows that the fluorescence intensity of the positive samples increased over 40 PCR

Table 1 List of thermal cyclers in the study "Eppendorf Mastercyclers Save Time and Cost in PCR"

Thermal cycler	Max block heating rate (°C/s)
Eppendorf MasterCycler nexus GSX1	5
Eppendorf MasterCycler nexus gradient	5
Applied Biosystem Veriti™ Fast	3
Life Technologies SimpliAmp™	4
Bio-Rad T100	4
"An Affordable and Portable Thermocycler for RealTime PCR Made of 3D-printed Parts and Off-the-Shelf Electronics" [6]	1.4
'Smartphone-based mobile digital PCR device for DNA quantitative analysis with high accuracy" [10]	4
Our system	1.4

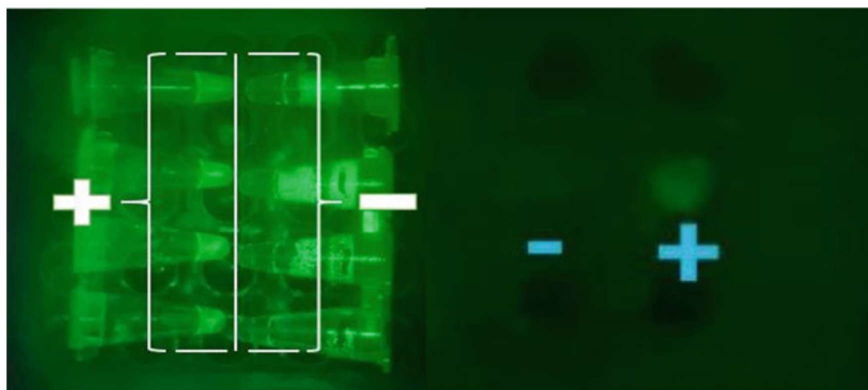

Fig. 11 Results images of tests, **a** 4 positive (+) and 4 negative (−) samples were put horizontally to test the ability to capture fluorescence differences, **b** 1 positive and 1 negative sample were put in the heat block with heated lid installed

cycles. Positive samples were also successfully amplified in the commercial PCR system (data not shown).

5 Conclusions

In this study, a highly integrated, low-cost, and portable realtime PCR system for genetic testing has been proposed and successfully built. The system achieved heating and cooling rate of 1.4 °C/s. The optical setup successfully detected fluorescence signal in realtime during the amplification process. We aim to increase the temperature ramp rate in future work by using a higher power Peltier module in combination

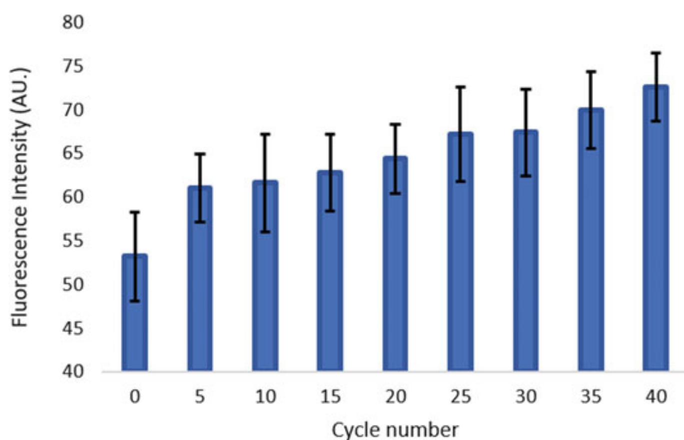

Fig. 12 Average fluorescence intensity of positive samples over 40 PCR cycles

with a better heat insulation design for the heat block. The optical setup design could also be improved to provide better contrast between negative samples and positive samples as well as lower variation in fluorescence intensity between replicates in a same run and in different runs.

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Conflict of Interest The authors declare that they have no conflict of interest.

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