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Proceeding Paper

Multiple Camera Fluorescence Detection for Real-Time PCR †

Seul-Bit-Na Koo [1,2], Hyeon-Gyu Chi [1,2], Ji-Sung Park [3], Jong-Dae Kim [1,2], Chan-Young Park [1,2], Yu-Seop Kim [1,2] and Deuk-Ju Lee [1,2,*]

[1] School of Software, Hallym University, Chuncheon 4252, Korea; rntmfqlcsk@gmail.com (S.-B.-N.K.); hyeongyuc96@gmail.com (H.-G.C.); kimjd @hallym.ac.kr (J.-D.K.); cypark@hallym.ac.kr (C.-Y.P.); yskim@hallym.ac.kr (Y.-S.K.)
[2] Bio-IT Research Center, Hallym University, Chuncheon 24252, Korea
[3] Biomedux, Suwon 16226, Korea; jspark@biomedux.com
* Correspondence: dlemrwn@hallym.ac.kr
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Abstract: The general polymerase chain reaction (PCR) amplifies DNA and analyzes the amplification results of the quantified DNA. Recently, real-time PCR has been developed to detect DNA amplification in various ways. The conventional camera-based system is too expensive and difficult to reduce device size. In this paper, we propose a low-cost, compact fluorescence detection system for real-time PCR systems using an open platform camera. To simplify the optics, four low-cost small cameras were fixedly placed, and the entire tube was divided into four quadrants to minimize the field of view. In addition, an effective image processing method was used to compensate. The proposed system measured the fluorescence detection performance on the basis of the amount of DNA using various fluorescent substances.

Keywords: real-time PCR; fluorescence detection; open platform; image processing; Raspberry Pi

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1. Introduction

The polymerase chain reaction is an important technique in biological research because it can check diseases by small amounts of DNA [1]. The DNA detection process consists of DNA extraction, DNA amplification, electrophoresis, and gel image analysis. However, PCR process is time consuming and labor intensive, with weak analysis throughput. To overcome these shortcomings, real-time PCR has been developed. The amount of nucleic acid amplification product can be detected in real time and analyzed in a shorter time than the conventional method. In general real-time PCR experiments, methods for detecting fluorescence include a method using a photodiode and a camera-based method [2]. The method of using a photodiode is typically measured after establishing an optical system using a dichroic mirror. However, compared to a general filter, it has a drawback, namely, that an optical distance for reflecting and refracting light is required. It also requires a lot of optical components and distances. Another detection method, the camera-based method, is expensive and large in size [3]. Recently, many open platforms have been developed and are used for image analysis after PCR detection. As high-performance smartphone cameras for open platforms continue to be developed, imaging devices are also rapidly developing [4]. With the development of CMOS sensor technology, manufacturing costs have been reduced, and ultra-compact cameras with excellent performance are being developed. Much research is being conducted to make small-sample processing and biochemical processes inexpensive and simple. Therefore, many research methods have been reported, but up to now, most devices use a real-time PCR device using a tube type. In order to obtain accurate experimental results when measuring fluorescence, images must be taken under the same conditions, and all tubes must be photographed and analyzed at once. In order to solve this disadvantage, if the tubes to be photographed at one time

are divided into several groups, and the images obtained from each can be synthesized into one, the real-time PCR apparatus can be compact and inexpensive. Therefore, in this paper, we propose a low-cost, compact fluorescence detection system for a real-time PCR system using an open platform camera. A case was made using parts that can be easily purchased on the market, and a self-made integrated board was used. In order to simplify the optical component during fluorescence measurement, four low-cost small cameras were fixedly placed, and the entire tube was divided into four quadrants to minimize the field of view. An effective image processing method was used to compensate for the reduction in the signal-to-noise ratio of the superimposed image. The proposed system used double distilled water (DDW) and fluorescent solution (FAM) to perform qualitative analysis and quantitative analysis comparisons. As a result of the experiment, the proposed system was able to obtain similar results with smaller size and lower cost than the existing system. Therefore, it can be applied to a system for measuring various fluorescence that requires a wide field of view.

2. Material and Methods

Figure 1a shows the overall structure diagram of the system proposed in this paper. It was composed of a dark room to prevent blocking and reflection of light. To efficiently measure the fluorescence of FAM, an LED, an excitation filter, an emission filter, and an open platform camera were included. At the bottom of the system is a black aluminum block and a 5 × 5 well that will hold 25 tubes. The excitation filter was mounted in front of the LED, and the reflected fluorescence on the tube was measured with an open platform camera through the emission filter. LEDs and camera hubs were connected to the Arduino and turned on only when shooting to prevent photobleaching. The height of the tube and the camera were fixed by setting the minimum focal length of 100 mm to enable the same fluorescence detection in 5 × 5 wells. Figure 1b shows the quadrant's illumination and imaging axes position of the quadrant to photograph all tubes under the same conditions. The green circles represent the 4 camera positions, and the blue squares represent the 4 LED positions. The 5 × 5 well was divided into 4 quadrants into 3 × 3 wells to minimize the optical complexity, and the camera and the LED were arranged. The translucent blue circle indicates the half angles of the LEDs in the first quadrant. Each of the yellow boxes shows an area of the four wells that stand out when shooting in each quadrant. These arrangements allow the entire tube to be viewed from any angle, resulting in a uniform, low distortion image. Since each quadrant contains more than 3 × 3 wells, the middle well was compensated for signal-to-noise ratio (SNR) reduction by replicating in all four quadrant images.

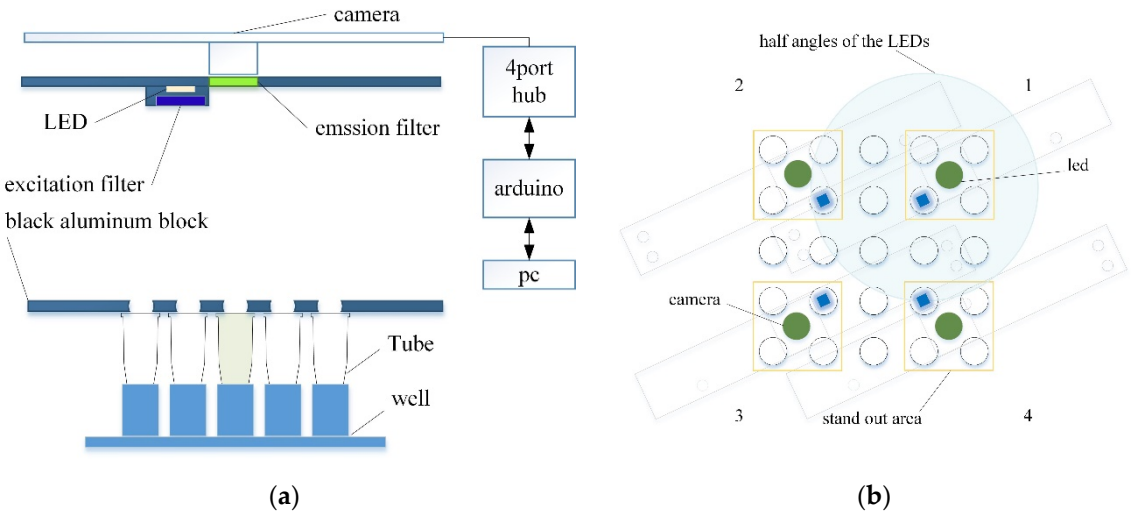


Figure 1. (**a**) System structure diagram; (**b**) 4 quadrants of all 5 × 5 wells: camera and LED location.

In order to stably fix the positions of 25 tubes in each quadrant, a mark sheet was made and then placed under an aluminum block well for calibration. Figure 2 shows the angle of the image rotation center used the relative position of the upper right end tube. Then, the angle between the center position of the 25 well and the x-axis of the image was calculated. For the experiment, FAM 5uMole and DDW, which are reference reagents similar to the fluorescence brightness after PCR amplification, were used. Each of the 25 wells was injected with 36 µL, and 4 images obtained by shooting were obtained as one integrated image through the above image processing process.

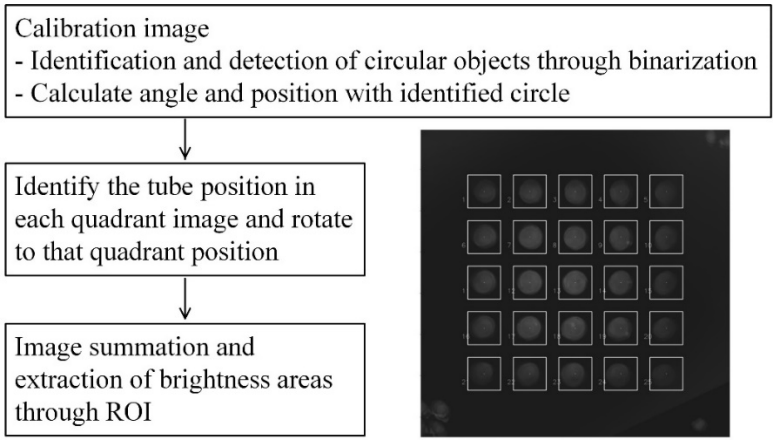
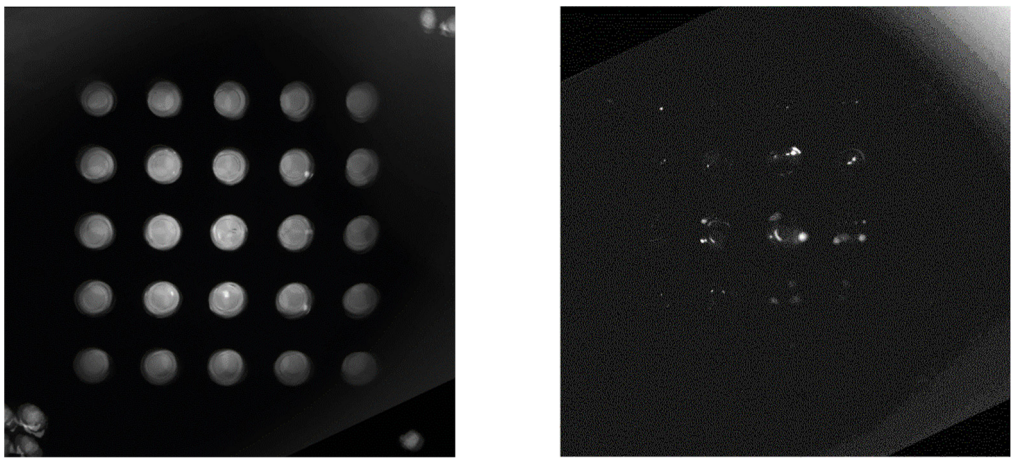


Figure 2. Image processing diagram and region of interest (ROI).

3. Results and Discussion

Figure 3 shows an image of four rotated images combined into one. Figure 3a is a FAM image and Figure 3b is a DDW image. The DDW image was amplified 6 times to improve visibility. Figure 3a,b shows the ideal match of the synthesized images.

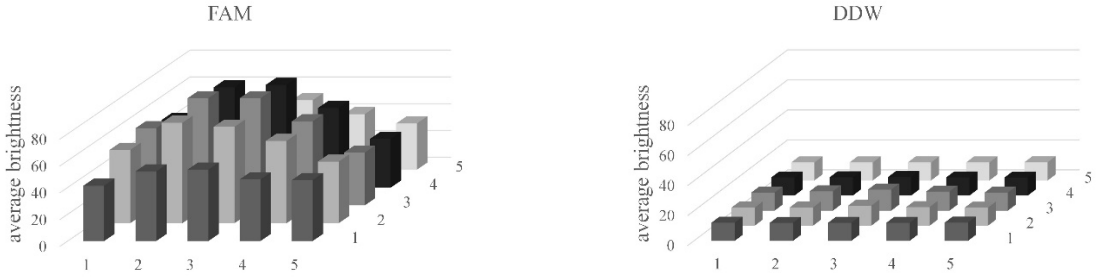


(**a**) (**b**)

Figure 3. The average brightness of each of the 25 tubes is shown: (**a**) FAM image; (**b**) DDW image.

Figure 4 shows the average brightness of each of the 25 tubes. The left graph shows the image of FAM and the right graph shows the image of DDW. It can be seen that the average brightness of the tube image containing DDW is around 12, and the average brightness of the tube image containing FAM is generally over 55. Figure 5 shows the relative gain difference between FAM and DDW. In fluorescence detection, the brightness through relative gain is based on the tube at the same location in each well. It is a value obtained by dividing the brightness of DDW by the difference in brightness of FAM and

DDW. Relative gains show more than 4 on average. Table 1 shows the average brightness and relative gain of tubes containing FAM and DDW.



(**a**) (**b**)

Figure 4. Average brightness of the tubes with (**a**) FAM solution and (**b**) DDW.

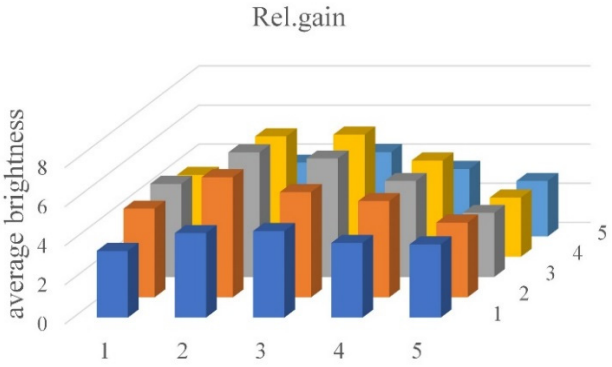


Figure 5. Relative gains.

Table 1. Brightness and relative gain statistics of each tube.

	FAM	DDW	Rel.gain
mean	55.23	12.27	4.48
min	34.48	12.00	2.84
max	85.11	14.03	6.37

Given that the relative difference in relative gain is compensated by calibration, it can be seen that fluorescence detection is possible using an inexpensive open platform camera. Therefore, this indicates the possibility of equipment capable of photographing a large number of wells without including complex optical elements.

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