Multiple Camera Fluorescence Detection for Real-Time PCR

Sciforum-043443

Seul-Bit-Na Koo, Hyeon-Gyu Chi, Ji-Sung Park, Jong-Dae Kim, Chan-Young Park, Yu-Seop Kim and, Deuk-Ju Lee
Department of Convergence Software, Hallym University
Bio-IT Research Center, Hallym University

abstract

In this paper, we propose a low-cost, compact fluorescence detection system for real-time PCR systems using open platforms 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 a fluorescent substance.

Background

- Real-time PCR (RTPCR):
  - RTPCR amplifies and quantifies target DNA molecules simultaneously using fluorescent dye.
  - Requires a lot of optical components and optical distance for reflecting and refracting light is required. expensive and large in size.
- Open Platform:
  - Development of backlight CMOS sensor technology has been developed, and manufacturing cost has been reduced, so that a very small camera with good performance suitable for a portable device has been developed.
- Present study :
  - We propose a detection system to achieve low cost and compactness in accordance with the above trends.
  - Propose a real-time fluorescence detection system that can a large number of wells at once with simple optical elements using multiple small open platform cameras.

Material and Method

Table 1 . Experiment set material

Classification	Detail	
Imaging system	IMX 179 Sony sensor 77.2˚ view-angle	
Fluorescence reagent	Probe type FAM (5 uMole/36㎕)	
Control reagent	DDW (Double Distilled Water)	
Filter	Excitation	CW: 470nm, BW: 40nm (Chroma Technology Corp.)
	Emission	CW: 535nm, BW: 50nm (Chroma Technology Corp.)
Light	White Led	240mA 120˚ view-angle

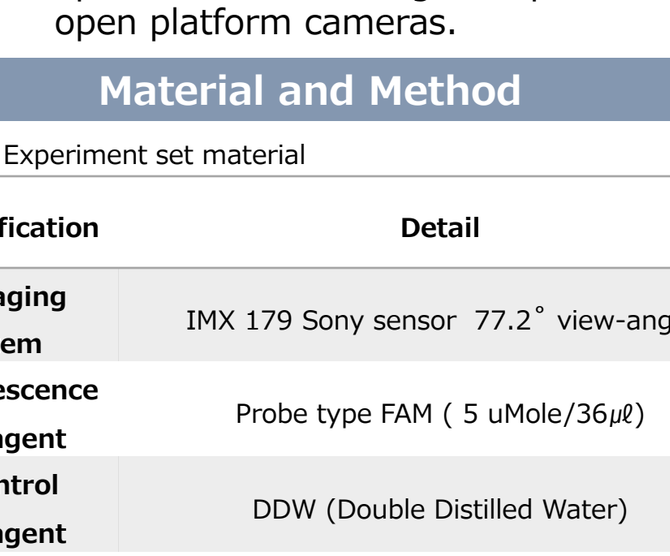


Fig. 1. Entire experimental system

- LEDs and camera hubs are 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 100mm to enable the same fluorescence detection in 5X5 wells.

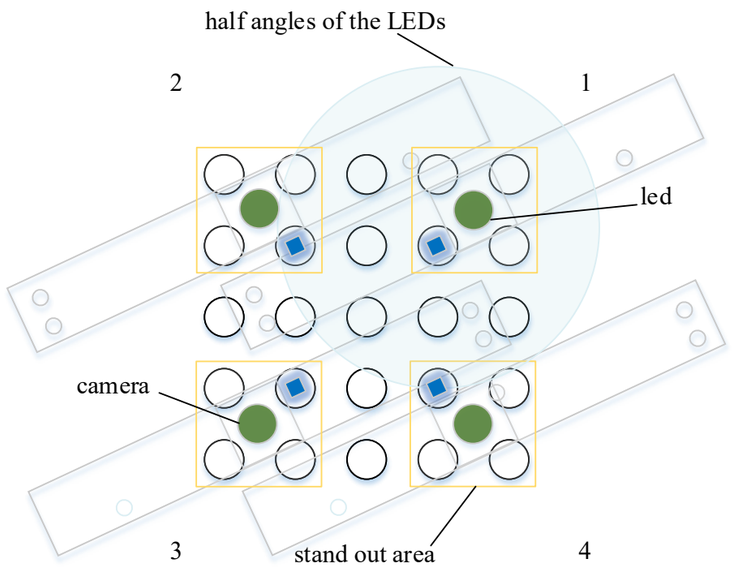


Fig. 2. quadrants of entire 5x5 well: Position of the illumination and imaging axes of the quadrant

- The 5X5 well was divided into 4 quadrants into 3X3 wells to minimize the optical complexity, and the camera and the LED were arranged.
- Since each quadrant contains more than 3x3 wells, the middle well was compensated for SNR(Signal to Noise Ratio) reduction by replicating in all four quadrant images.

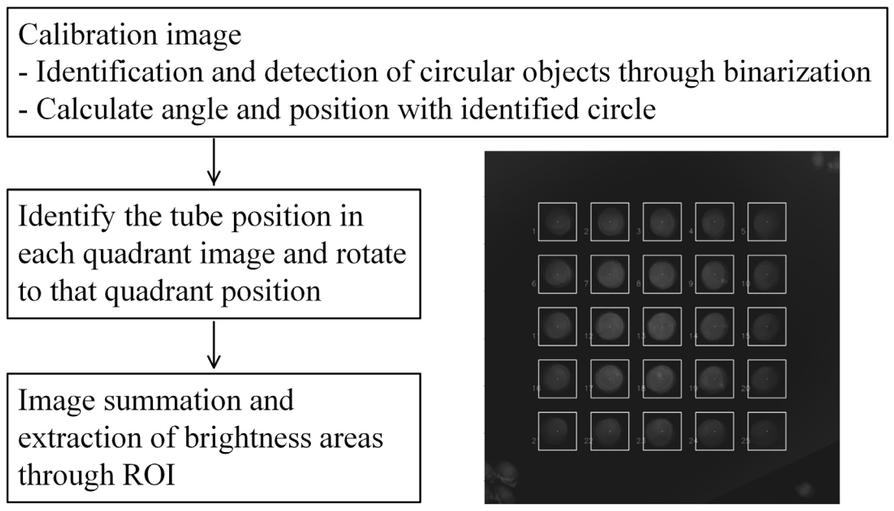
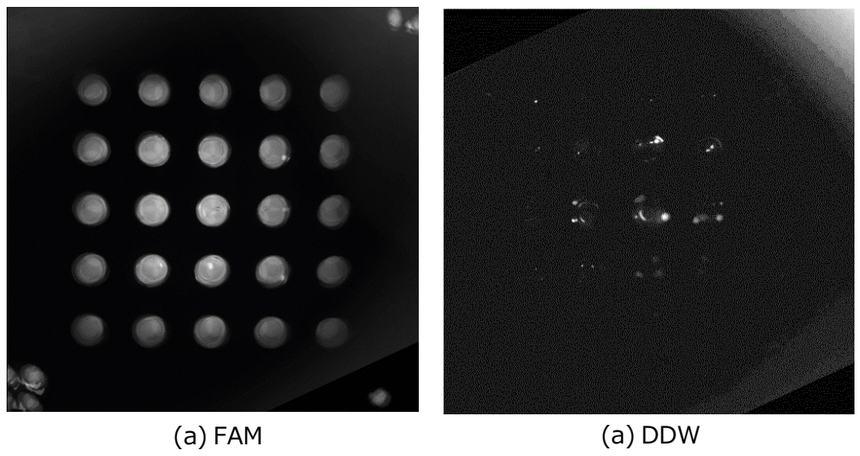


Fig. 3. Image processing diagram ROI (region of interest) processed image

- The angle of the image rotation center is calculated using the relative position of the upper-end tube in the upper right corner and the angle between the center position of the 25 well and the x-axis of the image.
- Reference reagents FAM 5uMole and DDW were injected into 25 tubes with 36ul each, and 4 images taken for the quadrant were acquired as one integrated image through image processing.

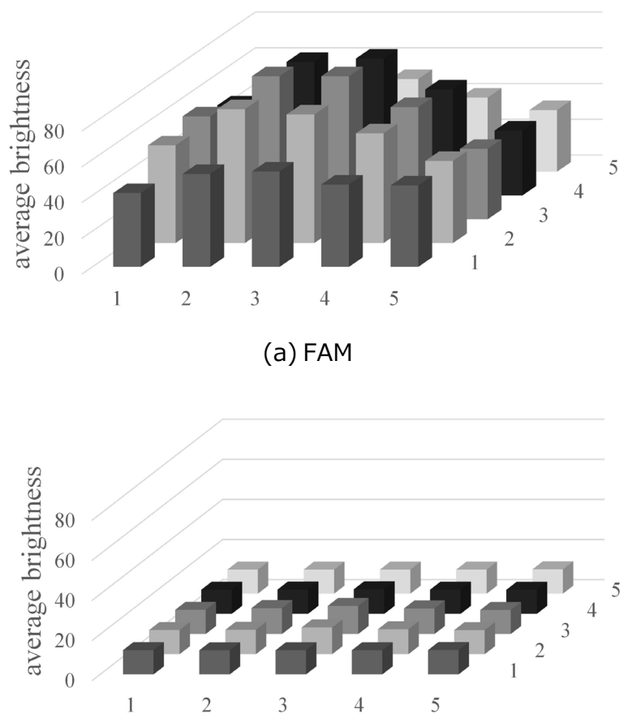
Result



(a) FAM (a) DDW

Fig. 4. Images synthesized from the quadrant images. The left image is for the FAM solution images and the right one is for the DDW images. The DDW images were enhanced by amplifying 6 times.

- The synthesized image showed a uniform match.



(a) FAM

(a) DDW

Fig. 5. The average brightness of each 25 tubes With (a) FAM and (b) DDW

- The average brightness of DDW is around 12, and the average brightness of FAM is generally over 55.

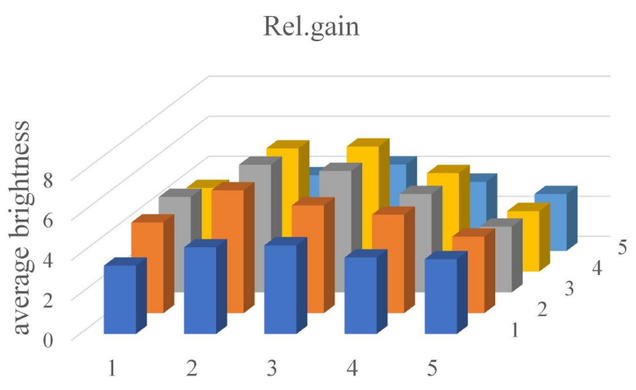


Fig. 6. Relative gains according to the well position

- The gap between the FAM and DDW brightness divided by the DDW brightness it is more than 4 overall.

Table 2 . Statistics of the brightness and relative gain 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	6.38

Conclusion & Discussion

- The small real-time PCR system made with only using the camera as an open platform except general essential optical was shown that capable of fluorescence detection.
- Given that the relative difference in relative gain is compensated for by the correction, it has been shown that fluorescence detection of a real-time PCR system with 5x5 wells is possible.

Reference

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