Cost-effective multiplex real-time PCR chip system using open platform camera

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OBJECTIVES

Commercial multiplexed real-time PCR

including photodiode and PCR tube

- Complex optical and thermal cycling structure
- long running cycling time

- Bulky and expensive
- Useable only large hospitals or laboratories

Proposed multiplexed real-time PCR

including open platform camera and PCR chip

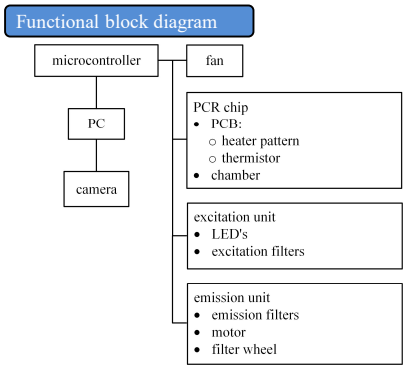
- Simple optics and thermal cycling structures
- Shortened execution cycling time

- More compact & low-cost
- Can be used anywhere

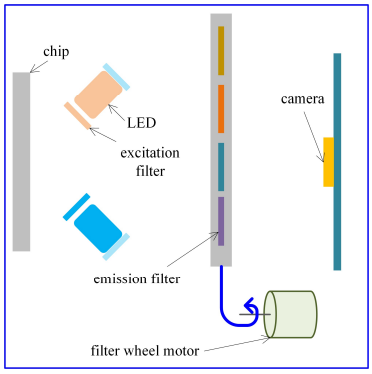
Suitable for POCT device

Material

Functional block diagram

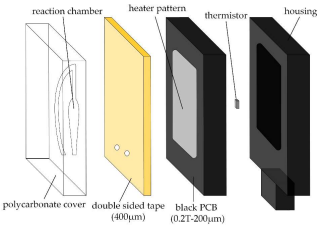


Structure of the proposed system



- **The color bands of the excitation and emission filters were selected to effectively detect HEX, ROX, CY5 and FAM dyes.**
- **PCR chip is placed vertically to the camera direction.**
- **The excitation unit illuminates the chamber with 45 degree lateral illumination**
- **Emission filters are operated by a filter wheel driven to linear servo motor.**

PCR chip



Reaction chamber

- polycarbonate
- optically transparent
- wide and thin

Black matte PCB

- Including heater pattern and thermistor

- suitable for brightness detection with a camera and higher brightness resolution.
- high-speed PCR thermal cycling

Method

Image processing flow to get ROI area

position of chamber in whole image → binarization(gray conversion, filtering, thresholding, morpholgical filterling) → ROI extraction by projection

Fluorescence verification experiment

single dye experiment	FAM	HEX	ROX	CY5	crosstalk experiment	FAM	HEX	ROX	CY5
DDW	X	X	X	X	ALL	O	O	O	O
FAM	O	X	X	X	FAM	X	O	O	O
HEX	X	O	X	X	HEX	O	X	O	O
ROX	X	X	O	X	ROX	O	O	X	O
CYT	X	X	X	O	CYT	O	O	O	X

- **The brightness of fluorescence of the plateau phase of PCR was emulated by 2 pico-mole/36μl dye solution for each fluorescence.**
- **The reagent was formulated that O is 2 pico-mole/36μl and X is 0 pico-mole/36 μl.**

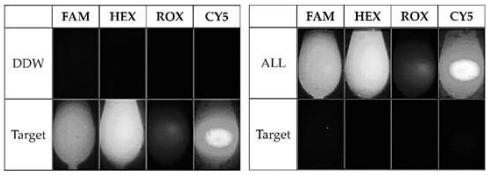
DNA amplification experiments

Reagent composition		
Reagents	Concentration	Volume
DNA(*Chlamydia trachomatis*)	6copy	5.4 μℓ
Master mix		18 μℓ
Primer mix	10 pmole/μℓ	9 μℓ
Distilled water		3.6 μℓ
Total		36 μℓ

Real-time PCR protocol			
Step	Temperature	Time	cycles
Pre-incubation	50° C	2 min	1
Pre-heating	95° C	10 min	
Denaturation	95° C	15 sec	40
Annealing	60° C	1 min	

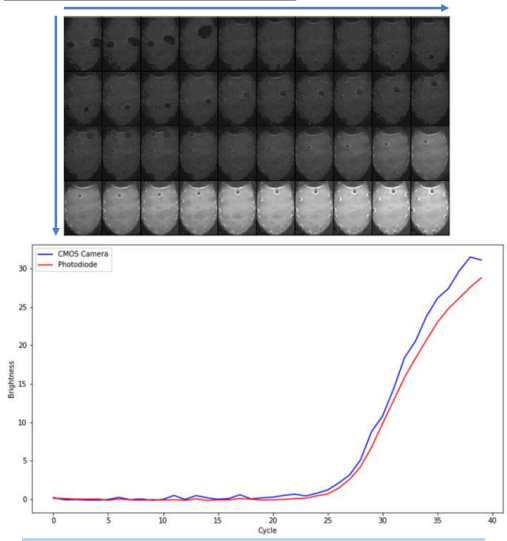
RESULTS

Fluorescence verification experiment



single dye experiment	FAM	HEX	ROX	CY5	crosstalk experiment	FAM	HEX	ROX	CY5
DDW	4.2	2.0	2.0	2.0	ALL	75.8	96.7	22.5	80.5
Target	62.1	95.3	21.1	71.2	Target	5.6	2.0	2.0	2.6
gap	57.9	93.3	19.1	69.2	gap	70.1	94.6	20.5	77.8
Relative gap	13.7	46.6	9.5	34.6	relative gap	12.4	45.3	10.2	28.8

DNA amplification experiments



- **Chamber images during 40 cycles of real-time PCR**
- **Mean brightness in ROI area of the proposed system (blue), amplification curve of the existing photodiode-based system(red)**

CONCLUSIONS

1. In the fluorescence verification experiment, the relative gap was more than 9.5 regardless of the presence or absence of dye, and the relative gap difference between the two experiments was within ±2.
2. In the amplification experiment, the difference in the cycle threshold value was within 1 as a result of quantitative analysis.
3. The results of single dye, dye crosstalk and actual DNA amplification experiments showed that the proposed system is sufficiently feasible as a POC multiplexed real-time PCR device.