ON-CHIP MELTING CURVE ANALYSIS
WITH A PRECISE TEMPERATURE COMPENSATION METHOD
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ABSTRACT
This paper presents a silicon-based integrated microsystem having a simple meander shaped micro reaction chamber, with external optical detection system for on-chip melting curve (MC) analysis with precise temperature compensation method.

KEYWORDS: Melting Curve Analysis, Temperature Compensation, Melting Temperature, Microfluidics

INTRODUCTION
MC analysis is an assessment of the dissociation-characteristics of double-stranded deoxyribonucleic acid (dsDNA) during heating, which is frequently used for microbiological identification [1] or for detecting genetic mutations [2], following real-time polymerase chain reaction (PCR) or reverse transcription-PCR (RT-PCR) processes. Hence, it is advantageous to implement on-chip MC analysis function on the integrated microsystem for nucleic acid (NA) based infectious disease diagnostics, together with real-time RT-PCR for viral RNA identification. Previous work [3] has demonstrated miniaturized MC analysis on the glass slides with nanoliter sample using external Peltier temperature controlling system, while this work presents a thermal unit integrated microdevice with precise temperature compensation technique, based on the melting temperature (MT) of given DNA sample.

DESIGN AND WORKING PRINCIPLE
Present microdevice for on-chip MC analysis (Fig.1) consists of four pairs of integrated microheaters and micro temperature sensors for temperature control of micro reaction chamber based on our group’s previous technology [4,5]. Experimental setup (Fig.2) has been established by assembling two different colored filters in the microscope, which has UV light source and an attached photo multiplier tube (PMT).

Figure 1: Photograph of the fabricated meander-shaped micro reaction chamber for on-chip melting curve analysis with integrated microheaters and micro temperature sensors.

Figure 2: Conceptual drawing of experimental setup with microscope having two different color filters – blue with UV light source for excitation and green with photo multiplier tubes for receiving.

Figure 3: Operational temperature setup with measured temperature profile for on-chip melting curve analysis.
Table 1. Measured MT of different samples from LightCyclerTM and integrated on-chip temperature sensor before compensation.

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<thead>
<tr>
<th>Test Methods</th>
<th>Melting Temperature</th>
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<tr>
<td></td>
<td>DEN2</td>
</tr>
<tr>
<td>LightCyclerTM</td>
<td>79.53°C</td>
</tr>
<tr>
<td>integrated on-chip</td>
<td>75.86°C</td>
</tr>
<tr>
<td>sensor</td>
<td>84.14°C</td>
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<td>both side turned on</td>
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<td>only one side turned on</td>
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Figure 3 shows the comparison between pre-set values and measured temperature profile. The fluorescence intensity (Fig.4(a)) has been measured as a function of voltage level from the oscilloscope connected to the PMT on the microscope. The first negative derivatives of fitted curve of measured fluorescence intensity shows MT of given DNA sample (Fig.4(b)).

EXPERIMENTAL RESULTS AND DISCUSSION

For verifying the measured on-chip MT result, same experiment is conducted using LightCyclerTM (Fig.5). However, it is found that there is a difference between the measured MT result from LightCyclerTM and the integrated microdevice (Table 1). To investigate the cause of this difference, we hypothesize that the measured temperature from the integrated micro temperature sensors could differ from the temperature of the micro reaction chamber, given that the MT of a particular DNA sample can be considered as an intrinsic material property, which do not vary according to the different measurement methods. To verify the hypothesis, relative temperature distribution of the microdevice is measured using IR camera. Even though IR camera images (Fig.6) could not give us the accurate temperature values, it is found that there is a severe temperature gradient on the surface of the microdevice. Simple linear fitting curve (Fig.7) of measured MT values is used to compensate the difference between the temperature of integrated micro temperature sensor and that of the micro reaction chamber. After compensation, we repeat the on-chip MC analysis and eventually achieve quite well agreed on-chip measured MT value with LightCyclerTM results (Table 2).

Figure 4: On-chip melting curve analysis result of RT-PCR product of dengue serotype 2 (DEN2) viral RNA: (a) voltage measurement from PMT, which represents the fluorescent intensity level with respect to the temperature; (b) its first negative derivative, which can pin-point MT of the given amplified dsDNA product.

Figure 5: Example of MT measurement of DEN2 RT-PCR product sample from LightCyclerTM, in which sharp peak of the first negative derivative of fluorescence intensity shows MT of 79.53°C.
Table 2. Measured MT of different samples from LightCyclerTM and integrated on-chip temperature sensor after compensation.

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<td>DEN2</td>
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<tr>
<td>LightCycler™</td>
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<tr>
<td>both side turned on</td>
<td>79.53°C</td>
</tr>
<tr>
<td>only one side turned on</td>
<td>79.15°C</td>
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Figure 6: Relative temperature distribution measured by IR-camera: (a) the case for both-side heaters in operation; (b) that for the single side heaters in operation. Color change from blue to white represents the relative temperature difference from cold to hot. White colored rectangular boxes in each images represents the boundary of the silicon micro reaction chamber.

Figure 7: Linear fitting of measured MT values from two different measurement method – LightCyclerTM and on-chip measurement for temperature difference compensation.

CONCLUSION
Consequently, we conclude that the present microdevice for on-chip MC analysis with precise temperature compensation method based on the MT value measurement has good potential for application in integrated NA-based diagnostics tools such as point-of-care microsystem for infectious disease diagnosis.

ACKNOWLEDGEMENTS
The authors would like to thank A*STAR for providing the financial support for this project.

REFERENCES

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