A CALIBRATION-FREE THERMAL DIGITAL MICROFLUIDIC DEVICE FOR ULTRAFAST DNA MELTING CURVE ANALYSIS

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ABSTRACT

A calibration-free thermal digital microfluidic (T-DMF) device was presented for ultrafast DNA melting curve analysis (MAC). The MCA over calibration-free T-DMF device can be finished in less than 7 seconds and clear enough to tell the difference between mutant type and wild type. This calibration-free method will be in great value for researchers who investigating the relative techniques and applications.

KEYWORDS: Digital microfluidics, DNA, Melting curve analysis.

INTRODUCTION

Our group has developed a thermal digital microfluidic (T-DMF) device and successfully finished DNA MCA on-chip in sub-7 seconds [1]. Nevertheless, calibrations of the on-chip temperature sensor were still entailed for each chip to correct the fabrication variation among chips. This process significantly brought down the throughput of the chip manufacture.

THEORY

We present a calibration-free T-DMF device for ultrafast DNA MCA as depicted Fig. 1. Instead of separating the thermal electrode into two parts, a heater and a temperature sensor, we utilize the heater alone with a constant input power to raise the temperature in samples. The temperature was highly related to the time of heating with a constant power input. That would simplify the DNA melting curve to fluorescence variation, depending on the time instead of temperature. Note that, there are three major factors influent the temperature respond: room temperature, droplet volume and the heating power.

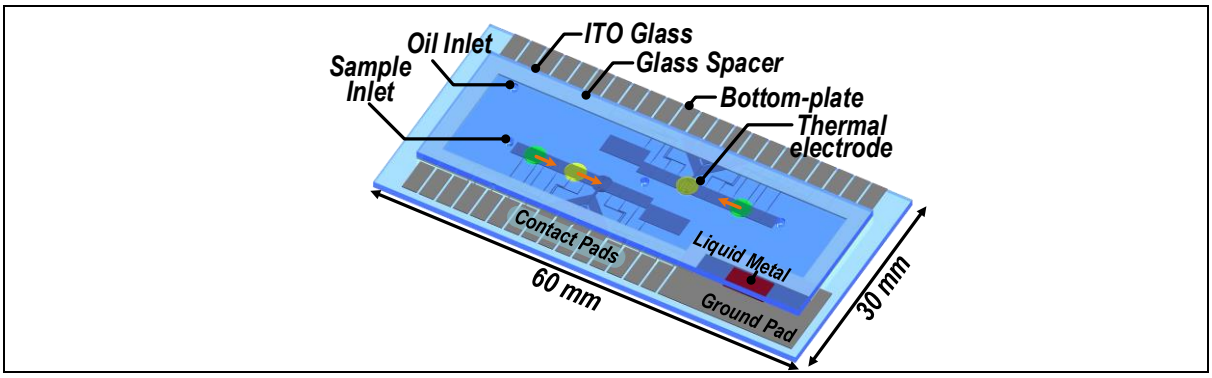

Figure 1: Proposed T-DMF device exploited for multi-droplet management and ultrafast DNA MCA.

EXPERIMENTAL

To address the issues, we built a MAX 4210 IC based regulator as a constant power source for the MCA. Engaged with an automatic droplet generating program, the droplet volume of the loaded sample was highly uniform. As shown in Figure 2, a serial of droplets with only 2.5% variation were generated automatically and heated by a constant power source. The temperature responds of the volume controlled droplets remain highly consistent.

RESULTS AND DISCUSSION

To demonstrate the performance of the calibration-free MCA, we took a DNA molecular beacon probe and its perfectly matched or mismatched targets as the model system for the ultrafast MCA and compared with its counterpart run in commercial qPCR machine (Fig. 3). The different between the

perfectly matched sample and mismatched sample can be easily distinguished on-chip. Note the testing time of the time consumption of the device case was only a couple of seconds, while the machine took an hour.

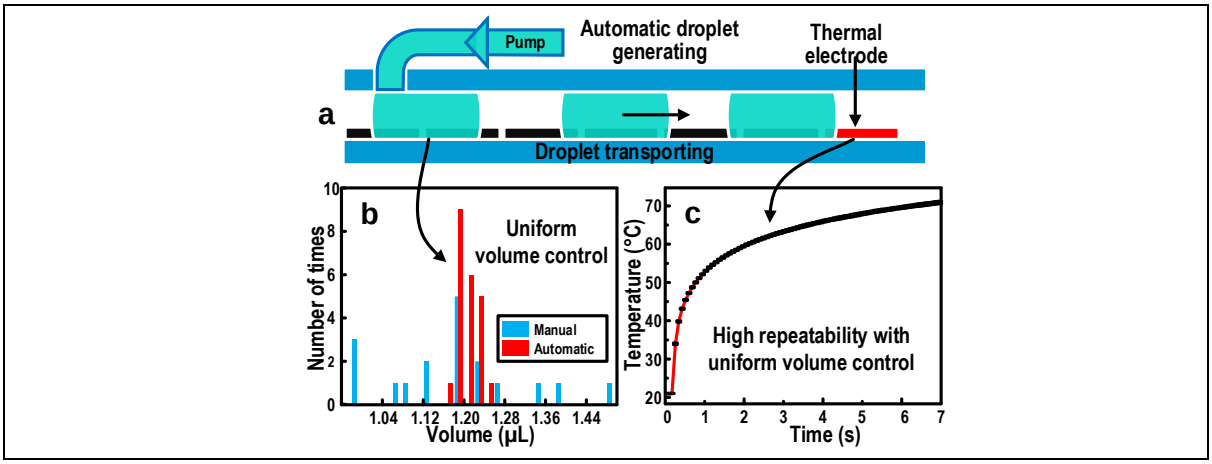

Figure 2: Automatic droplet generating and droplet heated under constant power source. (a) Droplets loaded and transported to the thermal electrode in serial. (b) Volume distribution of generated droplets under automatic program and manual operation. (c) The average temperature responding curve of droplets by charging the thermal electrode a constant power (0.4 W) at a room temperature of 21 ℃.

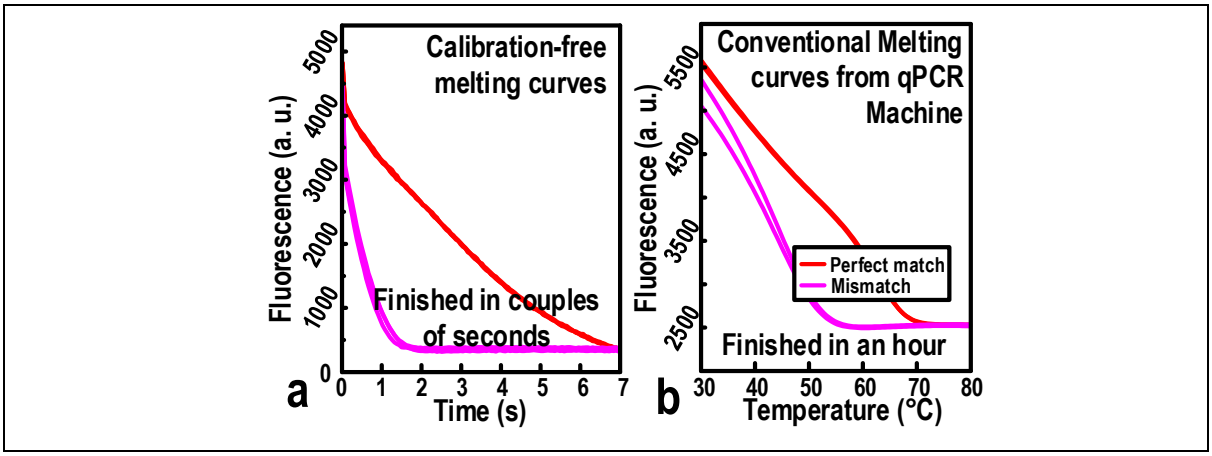

Figure 3: The calibration-free melting curves vs. the conventional melting curves. (a) Distinguishable difference between the perfectly matched samples melting curves and the mismatch samples melting curves under the calibration-free MCA. Note that, the time-consumption of such analysis takes only couple seconds which is over 100 times faster than the conventional one (b).

CONCLUSION

Such performance has greatly enhanced the practicability of T-DMF device for DNA analysis and paved the way to prompt low-cost clinical diagnostics.

ACKNOWLEDGEMENTS

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REFERENCES

[1] Chen, Tianlan, et al. "Sub-7-second genotyping of single-nucleotide polymorphism by high-resolution melting curve analysis on a thermal digital microfluidic device." Lab on a Chip 16.4 (2016): 743-752.

CONTACT

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