HIGH THROUGHPUT MELTING CURVE ANALYSIS IN MONOLITHIC SILICON-BASED MICROFLUIDIC DEVICE

J. B. W. Soon[1,†], P. Neuzil[1,2,‡], C. Fang[1], J. Reboud[1], C. C. Wong[3], and L. T. Kao[1]

[1]*Institute of Microelectronics, A*STAR (Agency for Science, Technology and Research), Singapore*

[2]*currently at KIST-Europe,Saarbruecken, Germany*

[3]*Division of Bioengineering, Nanyang Technological University, Singapore*

ABSTRACT

This paper reports the development of a silicon-based microfluidic device that enables ultra fast melting curve analysis (MCA) for genomic studies (DNA) and drug discovery (proteins). The system relies on the flow of nanoliter-sized discrete phase samples through a buried silicon channel. The samples are subjected to a temperature gradient generated by two external heaters along the channel. The channel also simultaneously serves as a light guide to illuminate the droplets and guide the emitted fluorescence to the detector outside the channel. The system provides a simple melting analysis of fluorescent-labeled molecular complexes, with high multiplexing capability and throughput. The chip-based solution is attractive due to nanolitre sized samples handling, resulting in fast heat transfer thus achieving ultra fast MCA for genomic studies and drug discoveries .

KEYWORDS: Melting curve analysis, Silicon channel, Microfluidics.

INTRODUCTION

Detecting DNA melting temperature through melting curve analysis (MCA) is a standard technique which is used to verify the specificity of the real-time PCR using a fluorescent intercalator such as SYBR-GREEN[1]. This procedure is typically performed after the PCR is completed in the real-time PCR instrument. More recently Ilija et al uses microfluidics device fabricated by xurography method to perform temperature gradient flow PCR [2], which even lower the cost in manufacturing this device. Generally a melting-curve analysis system (which is often a real-time PCR instrument) consists of a temperature controlled part, set of capillary tubes or wells, a light source, a filter set and a fluorescence detection unit, typically photo multiplier tube or CCD camera. Unfortunately, as of now these systems are bulky, costly and the melting curve is detected from rather large volumes of samples of more than a few µL. Performing statistics to increase the confidence requires large amount of proteins and drugs, thus increasing the cost of drug discovery. In drug discovery, differentiation in melting temperature when drug is applied indicates drug reaction on the protein unfolding [3]. Therefore it is of great interest to develop a simple tool that enables MCA at high-throughput for very low sample volume.

THEORY

A high-throughput system for ultra-fast MCA is proposed (Figure 1). The system and methods carried out will be an inexpensive alternative to currently used. The sample is injected as a plug (droplet or drumlet) into a flow of oil confined inside a capillary tube, where the oil flow rate determines the propagation speed of the sample, as the sample propagates through the capillary tube, which is subjected to a temperature gradient along it by using the PID controlled heating elements at both ends of the capillary, the florescence alters due to the temperature change in the droplet. At the same time the fluorescence is excited by a blue laser where its light beam is guided into the tube via a fluidic t-junction. Light interacts with the sample inside the capillary and the emitted fluorescence intensity and is detected at the other end of the capillary by a photomultiplier tube (PMT) while the fluorescence amplitude is recorded. An image of the real device integration is shown in Figure 5b. The silicon chip is mounted on a heater controller while a rapid prototyped interface is used to transfer samples in and out from the chip during the experiment.

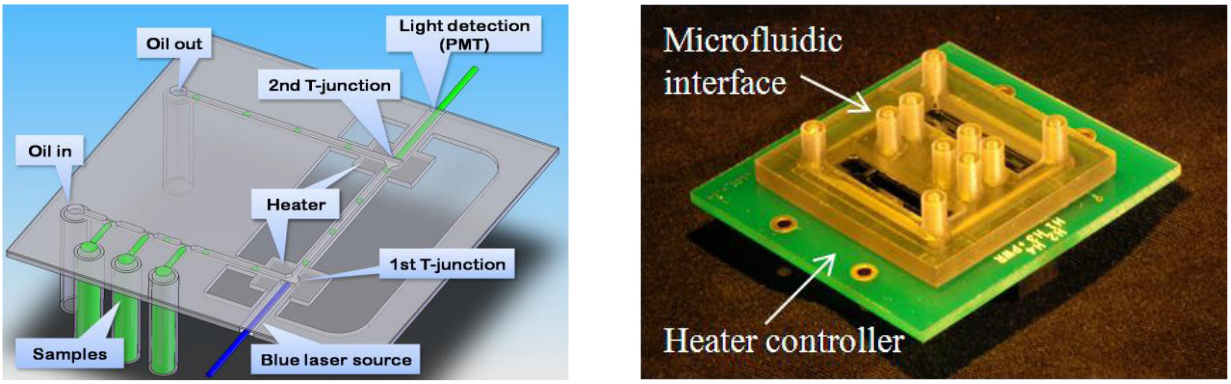

Figure 1: a) Schematics design of the silicon based microfludic chip. Droplet of a sample with double stranded DNA labeled by SYBR Green I enters through 1st T-junction and exits at 2nd T-junction subjected to a temperature gradient created by two heaters. The fluorophore is excited by the blue laser source and emission is detected by the photomultiplier tube (PMT). b) Complete device with microfludic interface.

EXPERIMENTAL

The fabrication process of the chip uses only single mask to create a monolithic silicon based microfluidic with 350µm diameter circular channel (see the process in Figure 2). The fabrication resulted in buried channels with nearly perfect circular cross-section. The fabrication begins with a deep reactive ion etching (DRIE) of a 350 µm deep and 25 µm wide trench using 1µm SiO_2 as a mask. This mask defined both channels as well as the inlet reservoirs. Then, 70 nm of thermal oxide (SiO_2) was thermally grown to protect the trench sidewalls. Subsequently the SiO_2 at the bottom of the trench was removed by anisotropic plasma etching to expose the Si substrate below while leaving the trench sidewalls protected. Next, isotropic etching is performed by XeF_2 vapor, resulting a nearly perfect circular profile channel forming at the bottom of the trench while keeping the integrity of the sidewall. At the same time, a 'donut' shaped pattern, design to allow the configuration of the inlet reservoir, indicated the completion of the circular channel, when the donut is fully released and fall off the chip. Later, the remaining SiO_2 was removed by buffered oxide etcher (BOE). As the last step, the channel walls were coated and simultaneously sealed by a parylene-C coating, creating an encapsulated buried channel in the Si chip. Figure 3 shows the cross-section scanning electron micrograph (SEM) of the buried channel and the inlet holes in the chip.

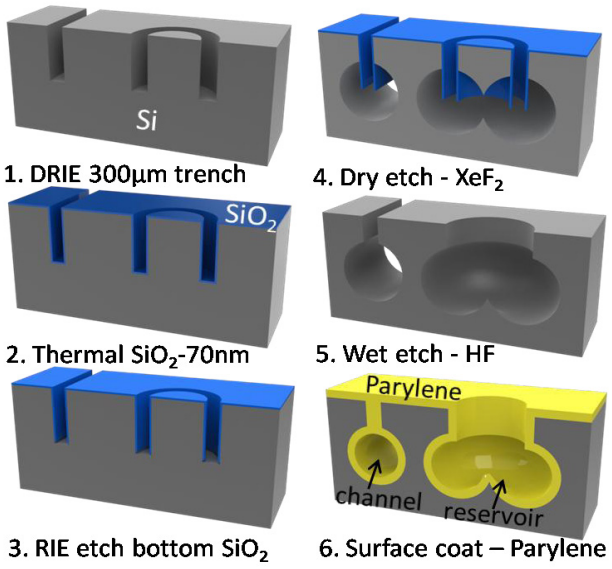

Figure 2: Fabrication process flow for microfluidic chip showing creation of encapsulated channel for DNA droplets' flow and reservoir for inlet or outlet of sample.

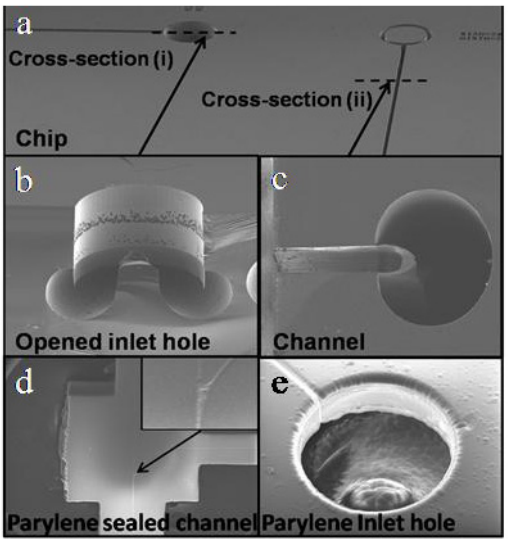

Figure 3: a) SEM photo of chip level showing one open inlet hole and the channel. b) cross section(i) showing the inlet hole. c) cross section(ii) showing the buried channel inside the silicon substrate. d) Channel encapsulation by parylene-C coating (inset) T-junction of sample mixing. e) Inlet of samples encapsulated by parylene-C.

RESULTS AND DISCUSSION

To demonstrate the applicability of inline optics for MCA, a scale-up system that resembles Figure 1 was built and the preliminary results on DNA in micro-liter droplets were shown in Figure 4. The evolution of the fluorescence intensity depicted the denaturation and renaturation of DNA duplex, with a melting temperature of 60°C (Figure 4a and b), which was further confirmed with the LightCycler (Roche).

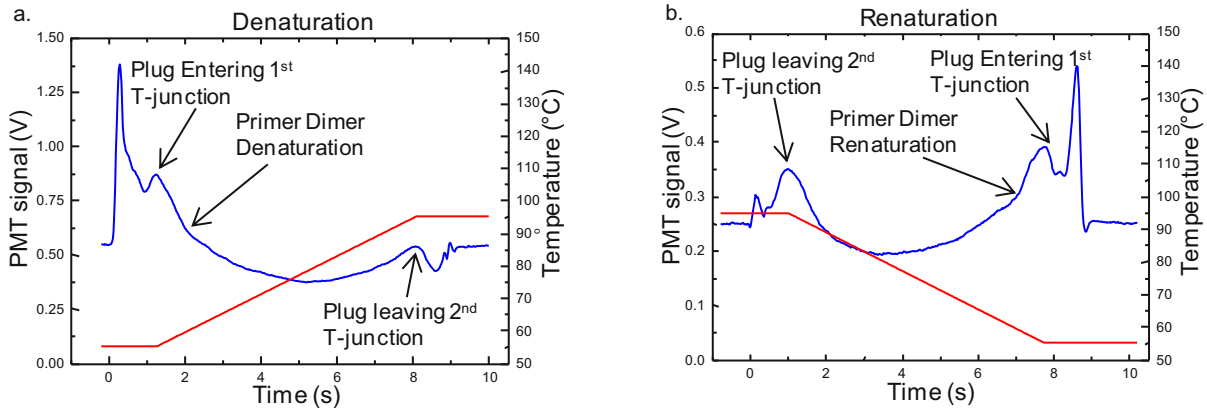

Figure 4: PMT detection of fluorescence in in-line optics a) MCA from 55°C to 95°C, showing denraturation at 60°C b) MCA from 95°C to 55°C showing a promising reversible process –renaturation of the same DNA at 60°C.

The previous results show a limited resolution of the melting point. A preliminary experiment to verify the heat transfer function was performed in time domain system in a virtual reaction chamber (VRC) as described [5]. The PMT output was plotted against the scanning temperature from 60°C to 100°C with temperature scan rate as a parameter (Figure 5). A sharp fluorescence reduction at the melting temperature(T_m) was observed. When the scanning temperature rate was increased from 0.1 °C/s to 5 °C/s, it is found that the melting point shifted, indicating a thermal lag due to heat transfer. From this, the correlation between temperature scan rate and the T_m shift gives the heat transfer rate between the heater and the sample. The heat transfer rate was determined to be 2.4 $°Cs^{-2}$. The same methodology can be applied in the flow through system by transforming time domain to space domain and the flow rate (effectively the temperature scanning rate) can be estimated, resolving a nominal flow rate to expedite future analysis.

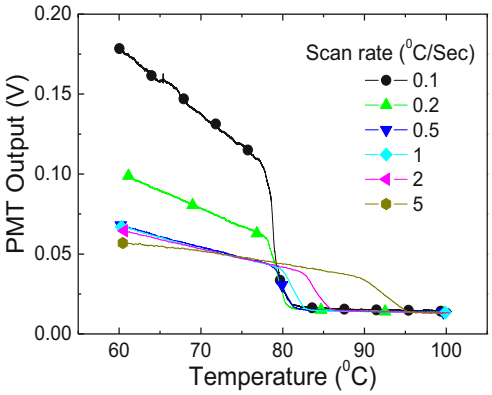

Figure 5: MCA of droplet based system with different temperature scanning rate, showing a heat transfer characteristics, can be used to determine heat transfer in the system.

CONCLUSION

The innovative use of inline optics coupled with nano-droplet flow in a self-aligned silicon light guide proposed here promises great potential for a rapid MCA system for drug discovery. By incorporating digital microfluidic functions upstream of the analysis, the system will provide a high multiplexing and could be extended to the recently reported DNA sequencing by denaturation [6].

ACKNOWLEDGEMENTS

The author wish to express their gratitude to Dr. Michael Entzeroth, Dr. Lee May Ann, A*STAR Experimental Therapeutic Centre, Singapore, for contributing the samples experimented in this project, for proofreading the script.

REFERENCES

[1] K. M. Ririe, R.P. Rasmussen, C.T. Wittwer, "Product differentiation by analysis of DNA melting curves during the polymerase chain reaction", *Anal. BioChem.*, **245**, 154-160, 1997

[2] I. Pješčić, C. Tranter, P. L. Hindmarsh, "Glass-coomposite prototyping for flow PCR with in situ DNA analysis", *Biomed. Microdevices*, **12**, 333-343, 2010

[3] W. D. Wilson, F. A. Tanious, M. Fernandez-Saiz and C. T. Rigl, Evaluation of Drug-Nucleic Interactions by Thermal Melting Curves, Methods in Molecular Biology, Drug-DNA Interaction Protocols, K R Fox Humana Press Inc , Totowa, NJ, 90, 219-240, (1998)

[4] D. M. Gaugel, CMOS-Compatible Fludic Chip Cooling Using Buried Channel Technology, Thesis, Carnegie Mellon University, (2001)

[5] P. Neuzil P, J. Pipper, and T.M. Hsieh, "Disposable real-time microPCR device: lab-on-a-chip at a low cost", *Molecular Biosystems*, **2**, 292-298, 2006

[6] Y. Chen, E. E. Roller, and X. Huang, "DNA Sequencing by Denaturation: Experimental proof of concept with an integrated fludic device", *Lab on a Chip*, **10**, 1153-1159, 2010

CONTACT

[‡]P. Neuzil, pavel@kist-europe.de;

[†]J. B. W. Soon, soonbw@ime.a-star.edu.sg