Real Time DNA Sequencing via Detection of Polymerization with Silicon based Pico-calorimeter chips

H. Esfandyarpour[1, 2], R.F.W. Pease[1], R.W. Davis[2, 3]

[1]Department of Electrical Engineering, Stanford University
Center for Integrated Systems, CISX B103, Stanford, CA, 94305
hesaam@stanford.edu, tel: (650) 723.4566 fax: (650) 723.4659
[2] Stanford Genome Technology Center
[3]Department of Biochemistry & Department of Genetics, Stanford University

For the past three decades, Sanger's method has been the primary DNA sequencing technology; however, inherent limitations in cost and complexity have limited its usage in personalized medicine and ecological studies.

It was shown that a new technology, Thermosequencing, could potentially reduce both the cost and complexity of DNA sequencing by using a picocalorimetric assay nanofabricated in microfluidic platform [1-6]. The described method relies on heat, IR and/or pH (which is correspond to charge or conductance modulation) detection resulted in DNA synthesis.

$$(DNA)_n + dNTP \xleftrightarrow{\text{DNA polymerase}} (DNA)_{n+1} + PPi + \Delta T + \Delta pH$$

To optimize the efficiency and fabrication of the technology, Finite Element Analysis was used to model the Thermosequencing system by simulating the DNA incorporation reaction series and the resulting product concentration and heat production. Different models of the platform were created to simulate the effects of the materials surrounding the system, to optimize the geometry of the system, and to concentrate reaction heat into specific regions for detection in the real system. The resulting concentrations of reaction products were used to calibrate the reaction speed and to design the heat sensors in the Thermosequencing technology. We recommend a modified structure for the detection platform and show how this new platform could dramatically improve the signal to noise ratio due to minimizing diffusion time (Fig. 1). We illustrate the design and experimental results (Fig. 2) of a primary template as well as different advantages and potential applications of the presented platform for DNA sequencing and genetics (Fig. 3).

The design rules for a silicon chip based devices as well as the surrounding platform module for liquid phase picocalorimetry are discussed. To increase heat production and for detection robustness, the amount of reaction, and consequently the amount of DNA on the bead must be increased. We investigated noise and common-mode rejection issues for sensor robustness and detection accuracy. Finally it will be discussed how the proposed micro-fabricated system is useful for a number of other bio-species detection and sorting templates.

References:

1. Esfandyarpour, H, Pease, RFW, Davis, EIPBN 2007.
2. Esfandyarpour, H, Pease, RFW, Davis, EIPBN 2008.
3.Esfandyarpour, H, Pease, RFW, Davis, RW, JVST B: Microelectronics and Nanometer Structures, 26 (2) pp. 661-665, 2008.
4. Esfandyarpour, H, et al., J. Biomicrofluidics, 2 (1), 2008.
5. Esfandyarpour, H, et al., IEEE Sensors Applications Symposium, Proceeding of, 12-14 Feb. 2008, pp. 89-94.
6.Esfandyarpour, H, Davis RW, NSTI-Nanotech, Vol.3, pp 422-425, 2007.
7.Esfandyarpour, H, et al., Int'l COMSOL Conference, Proc. 2007, pp-141-147.
8.Esfandyarpour, H, Davis RW, ICNMM2007, Proc. 2007, ref-3119.

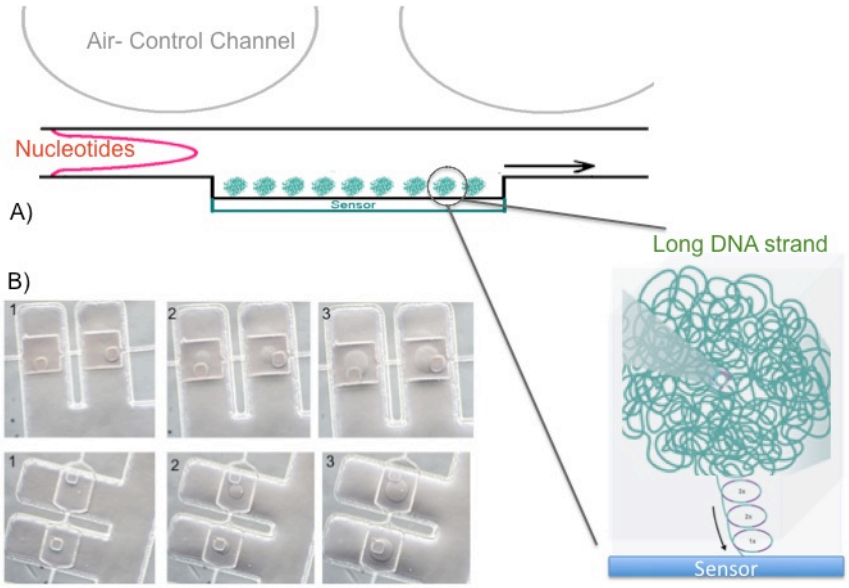

Figure 1. A) The schematic of optimized platform for DNA thermosequencer, which amplify SNR and minimize diffusion time of the system; B) Microfabricated PDMS-based gate controllable microchannels, three fabrication layer with unique wells under the channel structure (single channel controlling).

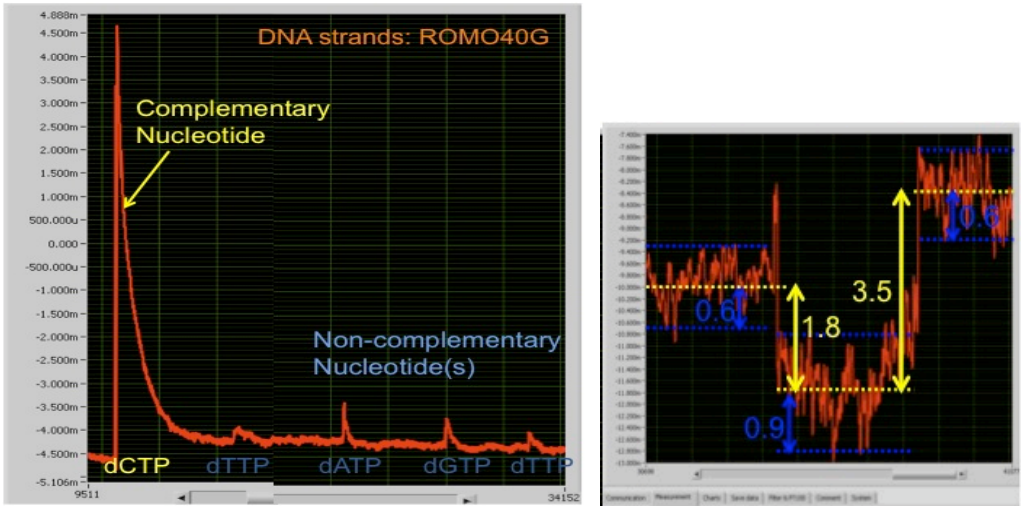

Figure 2. Initial experimental results from the thermopile-based thermosequencer; left: output signal from the sensor by injection of complimentary and noncomplimentary nucleotide(s). Right: The effect of light on the sensor results with Al/Si thermopiles on 4 um silicon membrane.

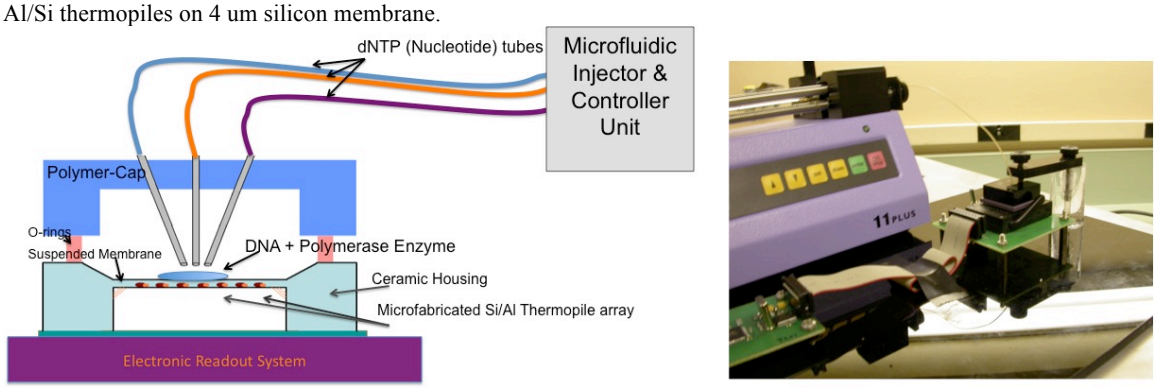

Figure 2. Left: Cartoon of the single-chip thermosequencer with Al/Si thermopile sensors. Right: It shows the experimental set up of the thermosequencer and its microfluidic and read-out platform.