Open-Loop, Rapid, Laser PCR System Using Transient Thermal Analysis, Optimization, and Environmental Control

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Introduction: The polymerase chain reaction (PCR) can be used to genetically detect specific pathogens [1]. The authors' previous work has shown the development of a microfluidic PCR system that uses an infrared laser diode to thermally cycle a 1 µl sample volume through 25 cycles in less than 12 minutes for the successful amplification of a 500 bp amplicon from λ-phage DNA [2]. This was accomplished using a disposable microfluidic chip and open-loop temperature control. This work presents substantial improvements to the aforementioned system that increases the amplification efficiency through more precise temperature and spatial control, and improved material compatibility.

Materials and Methods: To prevent bubble nucleation and mitigate evaporation in the microfluidic chip at the temperatures necessary for PCR nucleic acid denaturation, we have designed and implemented a nitrogen pressure manifold at 40 psi that seals against the chip ports. We have modeled and computationally optimized the open-loop control system described in [2] to compensate for the transient thermal dynamics associated with rapid thermal cycling (See Figure 1). A recirculating, water-cooled, copper heat sink has been designed and utilized surrounding both the chip for environmental control (σ=0.076ºC, 1 min), as well as the laser diode for power output stability (σ=2.4 mW at 430 mW, 2 min). Alignment pins were incorporated into the environmental control chamber to align the chip's sample volume to the laser diode to within 10 µm. Mineral oil encapsulation of the sample volume prevents deleterious interaction between the sample and chip surfaces.

Results and Discussion: Accurate and repeatable thermal cycles for the 3 discrete temperature holds required for PCR (denaturing at 94ºC, annealing at 68ºC, extension at 72ºC) are obtainable with this system design, as shown in Figure 1. Further, the resulting DNA target concentration after amplification, or the amplification efficiency, is improved from 10% to 120% relative to control (n=5).

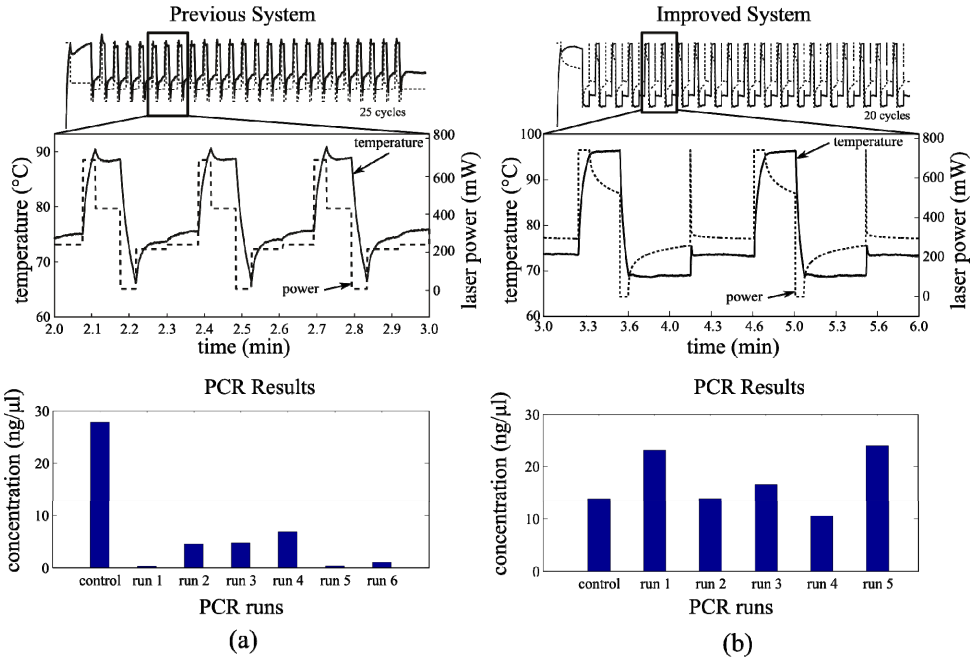

Figure 1: Laser power, microchip temperature profile, and amplification efficiency in the open-loop, rapid, laser PCR system (a) before and (b) after implementation of pressure and temperature controlled environment, oil encapsulation, and laser power optimization.

Conclusions: Improved open-loop control enables microfludic PCR in 12 minutes as a point-of-care genetic diagnostic tool with the same efficiency as conventional PCR systems.

References:

[1] Henderson D.A., Clinical Infectious Diseases, 2001, vol. 32:277–282.
[2] Pak N., Biomedical Microdevices, 2012, vol. 14:427–433.