CONTROL SCHEMES FOR MICROFLUIDIC VIRAL DNA/RNA AMPLIFICATION

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INTRODUCTION

The polymerase chain reaction (PCR) has become the standard technique for genetic detection and identification with applications ranging from clinical diagnostics to forensics [1-4]. PCR allows highly specific and sensitive detection of target sequences by exponentially amplifying a specific region of DNA or RNA from as little as a single copy through thermocycling a biochemical cocktail. While traditional PCR, based on either conductive or convective heating for the requisite thermocycling [5,6] has been an adequate tool thus far, we aim to decrease the cost and time associated with PCR by direct radiative heating of an aqueous sample in a polymer microfluidic chip. Our thermocycling technology relies on a 700 mW 1450 nm infrared laser diode for direct heating of the sample. A reaction volume of 1 μL, smaller than conventional reactions by 5-50x, decreases the consumption of costly reagents and allows for rapid cycling with heating rates 2-10x faster than conventional instruments. The reactions take place inside disposable polymer microchips that are directly micro-milled for accurate dimensions and repeatability [7]. Our lab has developed infrared radiation-based PCR thermocyclers with both open and closed-loop control schemes, all operated through user-friendly Labview interfaces. The different control methods each offer unique advantages suited to various applications, including rapid, quantitative PCR as well as high-throughput screening of multiple distinct genetic targets.

OPEN-LOOP CONTROL

A major challenge for implementing a low-cost polymer microchip for microfludic PCR is accurate temperature measurement. This often requires sensors in direct contact with the aqueous PCR sample, since the relatively low thermal conductivity of the microchip material prevents the device from reaching thermal equilibrium with its environment. This is especially true for a system using infrared heating, since heat transfer is localized to only the reaction volume. Another complication of direct contact between thermocouples and the aqueous PCR sample is interferance with the reaction chemistry. One way to avoid these challenges is to utilize open-loop control where there is no active temperature measurement requirement. A method for open-loop control offers the benefits of simple design, faster setup time, and elimination of contact temperature sensors while retaining the advantages of rapid radiative heating of a small volume. In order to obtain these benefits and implement open-loop control, excellent repeatability and accurate calibration prior to running PCR are crucial [8].

To ensure excellent repeatability, an environmental control fixture (shown in Fig. 1) was fabricated for mounting the microchip to achieve precise positioning and environmental stability. For positioning, the fixture aligned the microchip to the infrared laser using 1/16 inch dowel pins, which were press fit into a block that interfaces with the laser. Corresponding alignment holes were drilled and reamed into the microchips during manufacturing. For environmental stability, the fixture was machined from tellurium copper and features internal water cooling from a circulating bath to keep the area directly surrounding the chip at a known constant temperature, eliminating the effects of ambient temperature disturbances. Additionally, the fixture allows the delivery of 40 psig nitrogen to the fill ports of the microchip, enabling the chamber to remain pressurized during thermocycling to prevent the formation and expansion of disruptive air bubbles during the PCR reaction. To observe positioning repeatability, the system was mounted on an inverted microscope (Nikon, TE2000-E). An empty microchip was mounted in the fixture and imaged with the microscope, recording the position of the chamber. This process was then repeated for 10 trials. To test thermal stability, a micro-thermocouple (Physitemp, T240C) was

bonded into the microchip, which was filled with water, mounted into the system, and temperature was monitored for 1 min. These mechanical and thermal repeatability tests yielded an average variation in chip placement of 5 μm, or 1% of the reaction chamber width, and the standard deviation in reaction chamber temperature was found to be 0.076°C when maintaining a constant temperature.

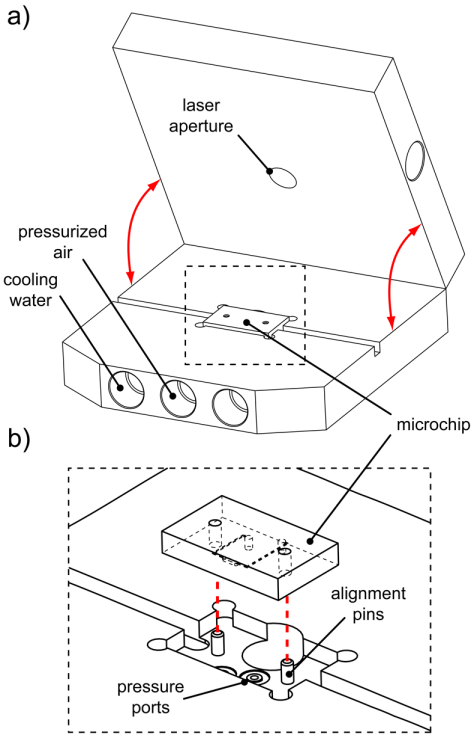

FIGURE 1. The environmental control fixture provides a mechanically and thermally repeatable environment for the microchip (inset) while allowing micrcope and laser access to the PCR sample contained in a chamber in the center of the microchip.

In order to implement open-loop control, a calibration relating laser driving voltage to aqueous solution temperature is necessary. Since the thermocouple used for calibration is heated directly by the laser, we compensated for this bias error as follows. First, it was known that the threshold voltage for the laser to turn on was 0.25 V. At this driving voltage the bias is zero and the thermocouple reads a temperature of 33°C, identical to the chamber temperature. Next, the driving voltage was increased as the reaction chamber was observed with a microscope. Boiling, as indicated by the rapid formation and expansion of bubbles in the chamber, occurred at 1.1 V and therefore corresponds to 100°C at atmospheric pressure. With the thermocouple centered and positioned at the bottom of the chamber, the measure bias increases from 0°C at 0.25 V to around 8°C at 1.1 V. The variables affecting this bias include thermocouple placement repeatability, bubble formation, and direction the thermocouple tip is bent. A calibration microchip was manufactured with the thermocouple bonded to the bottom layer (farthest from the laser). This prevents many of these variations between runs because the thermocouple tip is in the same position for every trial. Also, since our thermal modeling predicted that 90% of the radiation is absorbed in the first 0.5 mm of the aqueous solution, confounding effects of radiative heating of the thermocouple directly were minimized with the thermocouple located at the bottom of the chamber. A polynomial was fit to the bias corrected temperature measurements, resulting in a final calibration curve.

An optimal laser power profile for thermocycling was then determined using the calibration data along with calculations using an equivalent thermal circuit to model the heat conducted from the chamber. A lumped capacitance model is also included to model the transient temperature response due to the dynamic heating and cooling. An optimizer then modifies the laser power input to the circuit model to attain the desired three temperatures necessary for PCR: denaturing at 94°C, annealing at 68°C, and extension at 72°C. The performance of the calibrated system is shown in Fig. 2.

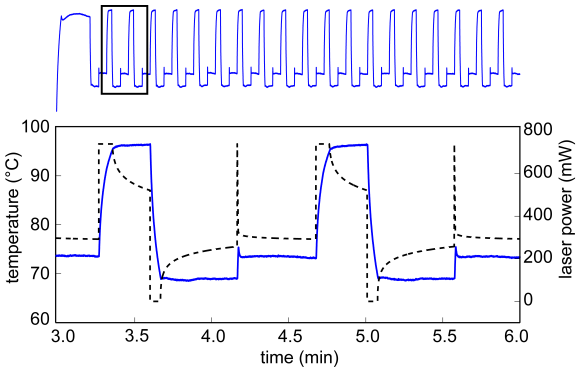

FIGURE 2. Using a calibration of laser power and reaction temperature combined with heat transfer modeling, an optimized laser power profile (dashed line) was generated for open-loop thermocycling.

The exponentially increasing and decreasing laser power profiles produced stable chamber temperatures and minimized the temperature undershoot and overshoot of the system. During one thermocycling run with 20 cycles, we measured the average standard deviation of the 20 respective denaturing, annealing, and extension steps to σ_d = 0.16°C, σ_a = 0.18°C, and σ_e = 0.15°C, respectively. In addition, the average heating and cooling rates of the system were 3.3°C/sec and 3.86°C/sec, respectively. To determine the run-to-run variability we measured the average denaturing, annealing, and extension temperatures over all 20 cycles for a run and compared these three numbers to another run. The average absolute difference between these temperatures was μ = 0.12 °C.

CLOSED-LOOP CONTROL

While open-loop control allows for a simplified instrument design and faster setup time, temperature feedback, or closed-loop control, can be useful for more complex aims such as indepdendenly controlling the temperature of multiple reaction chambers on the same microfludic chip. This is valuable for amplifying multiple different DNA sequences in parallel, requiring different annealing temperatures, simultaneously on the same chip from a single or multiple laser sources. Furthermore, closed-loop control allows for some variability in manufacturing and setup and accomodates disturbances from the environment.

In conventional convective or conductive thermocyclers, the common method of temperature measurement is to embed a thermocouple in the rough proximity of the sample container and allow the environment to thermally equilibriate. However, direct, rapid heating such as with laser radiation makes this method more challenging in that a very accurate model for the relation between solution temperature and measured thermocouple temperature is required.

To correlate the chip temperature with the solution temperature in our work, a calibration chip is used that has two thermocouples: one inside the reaction chamber and one embedded in the chip. By driving the laser at different powers and waiting for the calibration chip to reach steady state, a relationship is determined between the solution temperature and chip temperature. This relationship is then used in a real-time control algorithm for closed-loop control of the laser. A Labview program implementing this algorithm computes the difference between the measured and desired temperatures and uses a proportional-derivative (PD) controller to determine the laser power.

In the case of a single laser source, the beam is collimated and split with a lens array to focus radiation on adjacent chambers. A solenoid shutter is positioned below one of the chambers to attenuate the radiation hitting that chamber with actuation using pulse width modulation (PWM) control. By varying the duty cycle at which the solenoid is modulated, the temperature of that chamber can be effectively decreased up to about 10°C for chambers spaced 1 mm apart. For mutiple laser sources, a single laser driver operating them in tandem can be used with multiple independently controlled solenoid shutters. This case, dual lasers and shutters driven by a common laser driver, is depicted in Fig. 3.

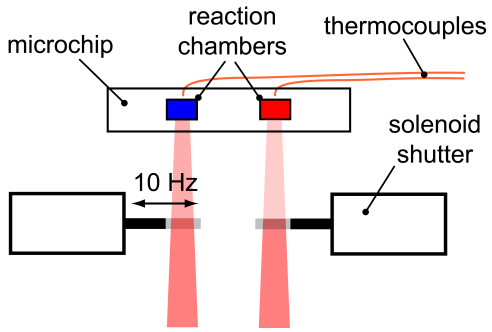

FIGURE 3. Infrared laser sources and solenoid shutters can be used with closed-loop control to perform PCR on multiple distinct targets simultaneously by monitoring temperature and independently modulating the laser beams.

The resulting capability of independent temperature control for two different reactions is shown in Fig. 4. By consolidating multiple thermocycling runs into a single step while using small sample volumes and harnessing the rapid direct heating by laser radiation, there is potential for massive cost and time savings. In addition, this configuration is scalable for increasing throughput by adding additional laser sources or dividing a single, more powerful laser and coupling each path with a shutter.

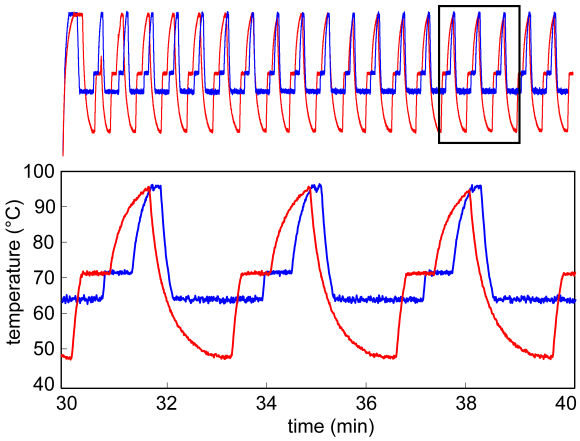

FIGURE 4. Closed-loop control and optical modulation enable independent temperature profiles for amplifying multiple genetic targets in a single insturment run.

CONCLUSION

The control schemes presented here coupled with the high-throughput, small volume, rapid cycling nature of infrared heating enable quick results in time sensitive applications. Open loop control is advantageous in that it offers simple and fast setup and execution, but it is most suitable for applications requiring only single reactions that are well calibrated. Open loop control is more complex and costly to implement and operate, but can allow multiple simultaneous chambers and accomodates environmental disturbances. Both open and closed-loop control have unique advantages, and the combination of a compact, portable system with rapid cycling times and disposable chips makes this technology ideal for point-of-care scenarios.

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