

# CONTROL SCHEMES FOR MICROFLUIDIC VIRAL DNA/RNA AMPLIFICATION

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## ABSTRACT

The polymerase chain reaction (PCR) has become the standard technique for pathogen detection and identification. By exponentially amplifying a specific region of DNA or RNA from as little as a single copy through thermocycling a biochemical cocktail, PCR allows highly specific and sensitive detection of target sequences. While traditional PCR, based on either conductive or convective heating for the requisite thermocycling, has been an adequate tool thus far, we aim to decrease the cost and time associated with PCR by direct radiative heating of the solution on a microfluidic chip. Our thermocyclers rely on a 700 mW 1450 nm infrared laser diode for direct heating of the aqueous solution. The sample volume of about 1  $\mu\text{L}$  decreases the use of costly reagent and allows for rapid cycling with heating rates on the order of  $60^\circ\text{C}/\text{s}$ . The reactions take place inside disposable polymer chips that are directly micro-milled for accurate dimensions and repeatability. Our lab has developed several infrared-based PCR thermocyclers with both open and closed-loop control schemes. Both control methods operate through custom made Labview programs that send and receive signals through a National Instruments data acquisition board (NI USB-6221). The different control methods have applications that include rapid, real time detection as well as high throughput screening of multiple different target sequences.

## OPEN-LOOP CONTROL

The benefits of open-loop control include the following: simple design, faster setup time, and elimination of contact temperature sensors. In order to obtain these benefits, calibration prior to running PCR and excellent repeatability are crucial [1].

To ensure this excellent repeatability for placement of our chips, a chip holder with a leaf spring and three points for full kinematic constraint in the x-y plane is used, as seen in

Fig. 1. The system was mounted on an inverted microscope (Nikon, TE2000-E) to test the repeatability. The error in the x-axis is insensitive because this axis is parallel to the reaction chamber's length. The standard deviation in the y-axis was found to be  $5.31\mu\text{m}$ , while that of the z-axis was less than  $16.4\mu\text{m}$ . From testing, these errors were found to be acceptable, with a standard deviation of solution temperature of  $0.1^\circ\text{C}$ .

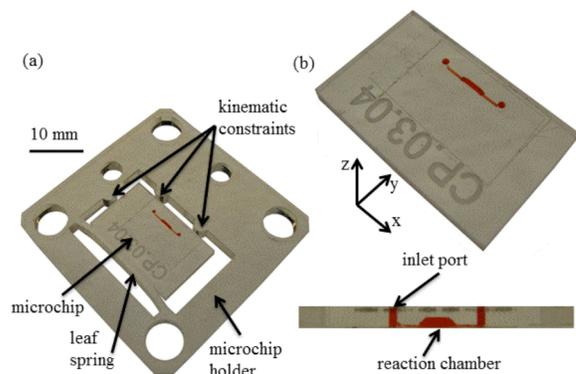


FIGURE 1. (a) Photograph of polymer chip holder showing kinematic constraints, leaf spring, and microchip, (b) and isometric view and side profile of polymer microchip.

Calibration of the relationship between laser power and solution temperature is the operating principle behind open-loop PCR. Given the excellent repeatability of the setup, a constant laser power results in a steady state temperature inside the chamber. It was known that the thermocouple used for calibration is directly heated by the laser radiation. This was taken into account by increasing the laser driving voltage from the turn on voltage of 0.25V to 1.1V which resulted in boiling. This laser power was noted as being equivalent to  $100^\circ\text{C}$ . This curve was used to create a simple open loop program to amplify  $\lambda$ -phage DNA. The program used the steady state voltages from the

calibration curve for the denaturing, annealing, and extension hold settings, and full laser power for the maximum ramping rate.

The benefits of a simpler system allow for the addition of other components. Specifically, current work is being done to incorporate the fluorescence detection capabilities of the microscope used for alignment to perform real time PCR. This system expands on the open-loop concept by introducing an environmental chamber to keep the area directly surrounding the chip at a known constant temperature. This allows for greater repeatability in the temperature profile because it eliminates ambient environmental disturbances. Furthermore, this system utilizes an optimized power profile for the laser that more accurately heats the solution. The optimal laser power is calculated 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 temperatures for denaturing, annealing, and extension. While the same calibration between laser power and solution temperature is used, thermal modeling is used for a much more advanced power profile that holds steady state temperatures with better accuracy.

### **CLOSED-LOOP CONTROL**

While open-loop control allows for a simplified system and faster setup time, temperature feedback is necessary for more complex systems. Specifically, if the size or shape of the reaction chamber changes, or if multiple chambers are present on the same chip, closed-loop control becomes preferable. Furthermore, closed-loop control allows for some variability in manufacturing and setup and rejects disturbances from the environment. The challenges with this control method result from difficulties in non-contact temperature measurement.

The approach used to measure temperature in our system relies on thermocouples embedded into the chips themselves. These thermocouples are isolated from the solution, so they do not interfere with the reaction chemistry, but measure the chip temperature close to the reaction chamber. To correlate the chip temperature with the solution temperature, 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 trend is determined between the solution temperature and chip temperature. This trend is then used in the program for closed-loop control of the laser. The Labview program operates by measuring the temperature and then determining which step of PCR it is in. The difference between the measured and desired temperature is calculated, and this is fed into a proportional-derivative (PD) controller to either increase or decrease the laser driving power. The PD controller maintains steady holding temperatures and also rejects ambient disturbances.

One of the applications of this closed-loop control scheme is a thermocycler that can amplify multiple different DNA sequences simultaneously on the same chip from the same laser source. The laser beam is split with a lens array to focus radiation on two adjacent chambers. Different reactions require unique annealing temperatures. Therefore, a solenoid shutter is positioned below one of the chambers to attenuate the radiation hitting that chamber. By varying the duty cycle at which the solenoid is operated at, the temperature of that chamber can be effectively decreased up to about 10°C. A temperature feedback loop allows for increasing the throughput of the thermocycler much easier than with an open-loop control scheme because the thermal crosstalk between adjacent chambers increases the difficulty of open-loop control exponentially.

### **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. 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.

### **REFERENCES**

- [1] N. Pak, C. Saunders, C.R. Phaneuf, C.R. Forest, *Plug-and-play infrared laser-mediated PCR in a microfluidic chip*, Biomedical Microdevices. (in press)