RAPID, INDEPENDENTLY CONTROLLED POLYMERASE CHAIN REACTION VIA MULTIPLEXED LASER RADIATION
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ABSTRACT
We report the design and testing of an instrument implementing thermal multiplexing for rapid microfluidic amplification of multiple DNA targets with different optimal annealing temperatures. Through spatial modulation of an infrared laser, distinct temperature profiles can be generated simultaneously in an array of microfluidic reaction chambers in a polymer device. For our dual-chamber system, temperature differences up to 15°C have been achieved. Such multiplexed temperature control has not been reported to date.

KEYWORDS: PCR, rapid, laser, multiplex, control

INTRODUCTION
Among the numerous efforts to increase throughput, speed, and affordability of genetic sample preparation, a promising approach is non-contact thermocycling using infrared radiation to perform the polymerase chain reaction (PCR) with small volumes on-chip [1] or in droplets suspended in oil [2,3]. There are several advantages of this approach. Existing PCR technologies, which range from conventional Peltier-based instruments to miniaturized devices with arrays of micro-scale chambers that interface with a heating block or feature resistive heaters [4], are limited to performing only a single reaction per run, since all samples are subjected to identical temperature setpoints. In cases where samples need to be screened for multiple target sequences that each require a different annealing temperature, current instrumentation solutions would still require separate runs for each reaction, regardless of the number of sample wells or enhanced ramping speeds. The traditional biochemical solution of multiplex PCR requires tedious primer design and suffers from reaction inefficiencies. Thus, there is a need for simultaneous control of multiple distinct amplifications in a fast, simple, and scalable system. The use of coherent infrared radiation and simple optics for temperature control allows the novel approach of thermal multiplexed PCR, providing individual reactions with optimal thermal conditions within a single run. This approach also offers fast ramping rates and compatibility with simple polymer microdevices for sample handling. Such a technology holds promise for new benchmarks in throughput, speed, and affordability.

THEORY
Thermal multiplexed PCR relies on key optical and heat transfer properties. An infrared laser was selected for its ability to heat water without damaging the substrate of the sample handling device; therefore, we use a wavelength of 1450nm that matches an absorption peak of water and a minimal absorption by common polymers such as poly-methyl methacrylate (PMMA) and polycarbonate. The use of a polymer for sample handling is not only beneficial for its affordability but necessary for its low thermal conductivity (e.g., 0.2 W/m·K), which is critical to both minimizing heat loss by conduction to the surrounding substrate and enabling thermal isolation of each reaction volume, i.e. minimal thermal crosstalk. Independent control of multiple reactions heated from a common source further requires the laser radiation to be divided and modulated. This modulation occurs during the annealing step, since reactions with different targets have unique primers with particular melting temperatures that correlate with Guanine-Cytosine (GC) content and length. The reaction with the highest annealing temperature will determine the baseline temperature maintained by the laser during this step. The shutters will then operate at a calibrated duty cycle to lower the other reactions to their unique annealing temperatures.

EXPERIMENTAL
The design of a prototype dual-chamber instrument was guided by optical and thermal modeling to predict the temperature response of aqueous reaction volumes in various substrates, geometries, and radiation sources [5]. The core elements include a 600mW 1450nm infrared laser diode [Hi-Tech Optoelectronics], an aspheric collimating lens [Thorlabs], a lenslet array fabricated in-house [6], a solenoid-driven optical shutter [7], and a polymer microchip with an array of 1μL chambers micromilled from cyclic olefin copolymer (COC) [8]. The configuration of the optical system and microchip is illustrated in Fig. 1. The microchip was designed to provide an adequate path length for absorbing radiation, as well as a relatively low surface-area-to-volume ratio to minimize both heat loss by conduction and adsorption of biomolecules to the walls of the device. The reaction chambers are spaced 1 mm center-to-center to align with the lenslets and air gaps are milled between the chambers for enhanced thermal isolation. The shutter is driven by a solenoid array salvaged from a dot matrix printhead. The solenoids are powered by an external 5V source, which is connected to a power transistor and triggered with a square-wave control signal generated in software and provided through a data acquisition device [National Instruments]. A thermocouple
Physitemp] is used to provide temperature feedback to the LabView control program, which uses a PID controller and outputs an analog voltage to modulate laser current. For running multiple reactions with different optimal annealing temperatures, the shutter is driven at 10Hz during the annealing step, during which it is operated at various duty cycles to attenuate the radiation.

Figure 1: Conceptual diagram (left) of the dual-chamber thermocycler with collimating and focusing optics for generating two focal spots and a solenoid-driven shutter for modulating the intensity incident on one of the chambers (inset). A generalized temperature profile (right) illustrates the common setpoints for denaturing (1) and extension (3) and the thermal multiplexing introduced by the shutter modulation during annealing (2).

RESULTS AND DISCUSSION
Characterization and demonstration of thermal multiplexing for dual-chamber system is shown in Figure 2. For a particular steady state annealing temperature, a second annealing temperature of 8-12°C lower can be achieved within the practical range of temperatures. This performance should prove capable of handling a variety of reaction pairs and will illustrate the advantage of optimal annealing temperatures for maximum sensitivity and specificity. Future work will focus on the application of this temperature modulation method to independently control multiple amplifications using a multiplex reactions designed to reflect temperature accuracy by exhibiting a range of amplification bias for a corresponding range of annealing temperatures [9]. This system is a proof-of-concept for a thermal multiplexing instrument scaled to 16 channels for rapid screening of viruses and bacteria at the CDC.

Figure 2: Experimental results for a) characterizing maximum temperature difference, or thermal crosstalk, \( \Delta T \), achievable between adjacent chambers vs. steady state temperature in one chamber and b) PCR temperature profiles for adjacent reaction chambers held at different annealing temperatures.

CONCLUSION
This combination of small reaction volumes (1\( \mu \)L), independent temperature control (\( \Delta T \leq 15^\circ C \)), high speed ramping (up to 60°C/s), and a low-cost, disposable microchip platform ($0.25/chip) has not been presented prior and represents a paradigm shift for PCR workflow, enabling potentially greater sensitivity and dramatically shorter processing times by consolidating multiple, unique reactions to be performed simultaneously at optimal conditions.
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