IR THERMOCYCLER FOR CENTRIFUGAL MICROFLUIDIC PLATFORM WITH DIRECT ON–DISK WIRELESS TEMPERATURE MEASUREMENT SYSTEM

J. Burger[1], André Gross[1], Daniel Mark[3], Felix von Stetten[1,3], Roland Zengerle[1,2,3] and Günter Roth[1,2,3]
[1] Laboratory for MEMS Applications, Department of Microsystems Engineering - IMTEK, University of Freiburg, Georges-Koehler-Allee 106, D-79110 Freiburg, Germany
[2] Centre for Biological Signalling Studies – BIOSS, University of Freiburg, Germany
[3] HSG-IMIT, Wilhelm-Schickard-Straße 10, D-78052 Villingen-Schwenningen, Germany

ABSTRACT

We present an infrared (IR) thermocycler with closed loop temperature control for performing fast polymerase chain reactions (PCR) in centrifugal microfluidics [1]. It consists of an IR ring heater and an on-disk wireless temperature sensor module with a resolution of 0.1 K [2]. The closed loop system enables to precisely control the temperature of the reagents even at varying conditions e.g. manufacturing tolerances of the polymer film disks [3], different locations of the cavities, ambient temperature changes. Due to the direct heating of the reagents by IR absorption we achieve fast average heating gradients of up to 4 K/s. Average cooling gradients so far are limited to 1.3 K/s. Our system is superior in terms of energy efficiency, temperature accuracy and overall reproducibility and robustness.

KEYWORDS

Lab-on-a-Chip (LoaC), centrifugal microfluidics, Polymerase Chain Reaction (PCR), Immunoassay, thermocycling, wireless temperature measurement

INTRODUCTION

In centrifugal microfluidic lab-on-a-chip systems both for the processing of immunoassays [4] as well as PCR [5] the tempering of the reagents is essential. These reagents are generally kept in cavities made of polymers with volumes of few microliters. Polymer cartridges with microfluidic control structures and cavities could be either injection-moulded or blow-moulded of films (figure 1). For tempering the reagents energy has to pass the cavity boundaries. Therefore the design of the cavity considering the material e.g. physical parameters - heat conductance, transmission spectrum and the geometry e.g. thickness of cavity walls has to be chosen appropriately. The reagents temperature depends on the amount of transmitted energy. It further depends on the reagents itself and the cavities/cartridges heat capacities. Our aim is to realize a robust and precise temperature control having an accuracy of 0.1 K independent on varying reagent volumes, production tolerances of the polymer cavities or changes in the ambient temperature. This is demanding both, measuring the reagents temperature directly in the cavity and controlling the energy supply accordingly. State-of-the-art centrifugal platforms [6] are tempering the reagents by circulating air. The temperature control is relying upon the temperature measurement of the air and the accuracy of the underlying models of heat transfer into the reagent cavities as well as out of the reagent cavities. Geometrical variations of the polymer cavity, changing cavity positions, production tolerances or inaccuracies of the reagents volume diminish the compliance of the model and hence potentially deteriorate the performance and especially the reproducibility of the assay or the PCR.

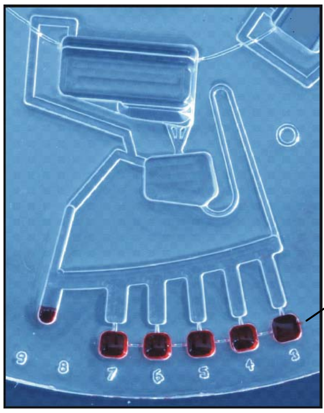

Figure 1: Blow-moulded polymer film cartridge with microfluidic control structures and reagent cavities (red) [7].

Directly measuring the reagent temperature enables to use direct heating systems. The process of transmitting the heat energy by convection to the polymer cavity walls and by thermal conductance through the polymer to the fluidic reagent is replaced by electromagnetic radiation. Microwaves would be an option [8] however we employ an easy controllable IR radiation source allowing to be handled without considerable safety restrictions. Thus the reagents in the microfluidic cavities are heated mainly by absorption of the IR radiation energy. The efficiency factor depends on various parameters e.g. the ratio of radiation over electrical energy of the IR heater, the optical spectrum of the IR heater, the geometrical optics to focus the radiation, the total absorption rate of the surrounding air. Especially the transmission spectrum of the lid material of the microfluidic cartridge and its thickness is of high impact. PCR thermocycling requires fast periodic heating to the denaturation temperature of ~92 °C as well as fast cooling of the reagent down to the primer annealing temperature of ~55 °C [5]. It is realized by a ventilator venting air of room temperature into the housing surrounding the microfluidic disk. Due to the air flow itself and the rotation of the centrifugal microfluidic cartridge the PCR reagents in the cavities are cooled by forced air convection. Both the IR ring heater and the ventilators are controlled by an embedded adaptive PID controller. Simulation studies with an electrical model analogous to the thermal network [9] assist in analyzing the theoretical aspects of heat transfer by radiation and energy flow by conductance/ convection.

SYSTEM SPECIFICATION AND DESIGN

Average heating and cooling rates of > 2 K/s should be achieved. The reagent temperatures in the cavities subject to the tempering or thermocycling process should be approached without significant overshooting and stable at the setpoint within a tolerance of +/- 0.2 K.

Heating method

For heating the reagents we choose an IR ring heater of type Omega IR beamer 2D [10] with an electrical power of 1700 W and a peak wavelength of ~2 µm as IR radiation source (figure 2).

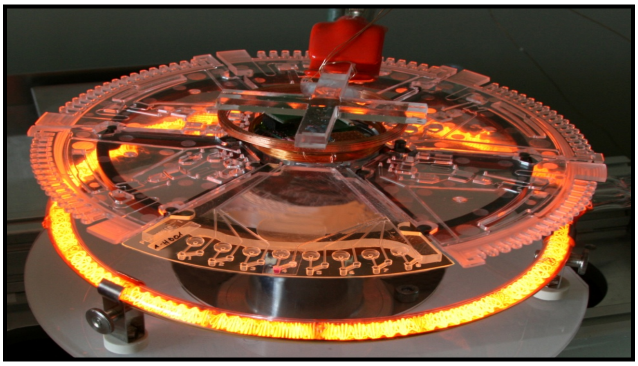

Figure 2: IR ring heater with on-disk temperature measurement system – first prototype.

This ring radiator with an inner diameter of 170 mm is fixed in the lid of the housing of a centrifugal analyser (figure 3). Half of the ring shaped glass body is gold plated such that the radiation emitted by the electrical coil of 560 mm length is focused to the reagent cavities. The coil is powered by a 230 V amplifier controlled by the temperature controller.

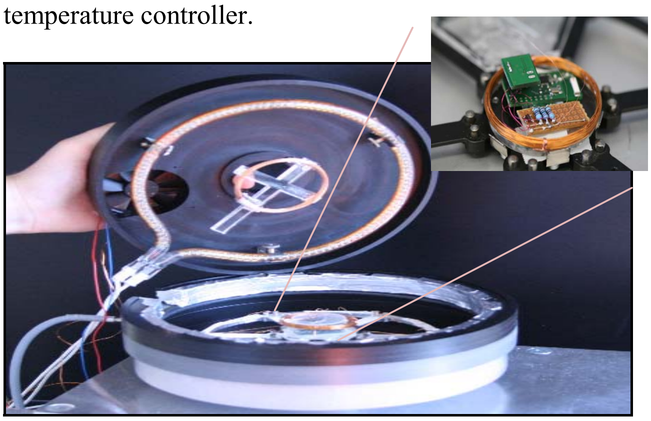

Figure 3: Closed system with IR heater, ventilator and temperature measurement system on rotary platform.

Cooling method

A ventilator of 7.7 W is mounted on the lid of the thermocycler (figure 3). Thus the air heated by absorption of the IR radiation as well as thermal conductance and convection from the glass tube of the ring heater can be vented prior to cooling by forced convection. The theoretical aspects of thermal energy transfer are illustrated in figure 4. The absorption of the IR radiation in the aqueous reagent decreases with depth. However due to natural convection there is a rather homogenous temperature in the reagent cavity. A small boundary layer to the COC results from the thermal conductance. A linear temperature gradient in the COC cavity wall follows a broader boundary layer to the surrounding air representing the forced convection of energy due to the rotating disk and the ventilated air flow.

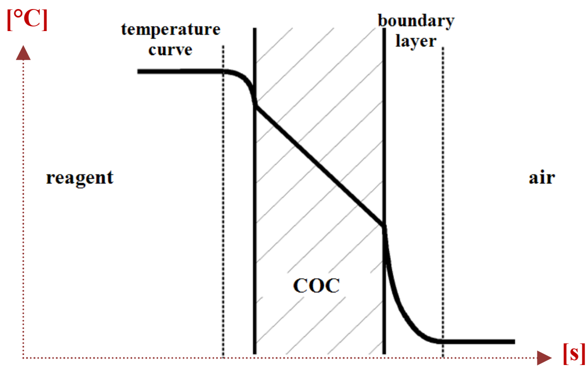

Figure 4: Cooling of reagent in microfluidic polymer cavity by conductance and forced convection.

Simulation studies by an analogous electrical model as R-C network (figure 5) are performed with LTspice [11]. Resistors represent the inverse of the thermal heat conductance or convection, capacitors represent thermal energy stores.

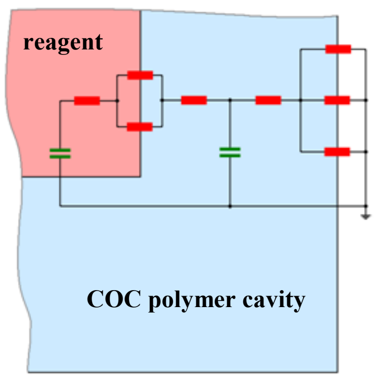

Figure 5: Analogous electrical model of heat energy transfer from reagent through polymer cavity wall to surrounding air by heat conductance, convection and radiation.

This way the heat flow (current) and the resulting temperatures (voltage) at any node as a function of temperature and time can be calculated.

Temperature measurement system

A carrier for the COC polymer film disk (Ø 210 mm) sealed by a COC polymer film of 150 µm thickness is connected to a motor spindle. The rotating components of the temperature measurement system (figure 3, top right) for data acquisition, A/D conversion and wireless transmission are fixed in the centre of this carrier (fig. 6).

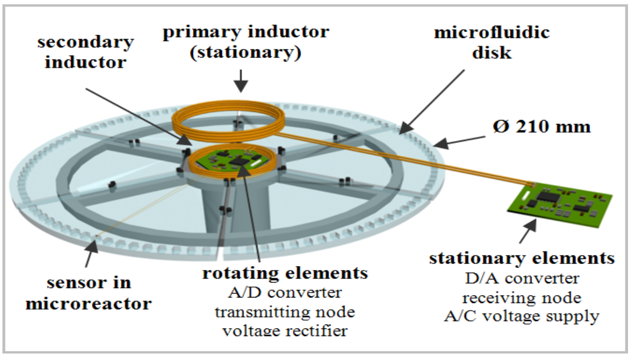

Figure 6: Setup with wireless data transfer / inductive coupling.

A NTC (negative temperature coefficient) thermistor [2] used as temperature sensor is placed directly in one of the cavities of the polymer film cartridge (figure 7). This SMD sensor of size of 0.6 x 0.3 x 0.3 mm³ is connected by isolated copper wires of 50 µm core diameter to an A/D converter [2]. Analogue signals scaled to 270 mV – 1500 mV are digitalized at a sampling frequency of 200 kHz with a resolution of 10 bit leading to digital values of 0 - 1023. This digital signal is transmitted wirelessly by a 2.45 GHz transceiver [2] to a stationary receiving node at a data rate of 100 Hz. Both the transmitting and receiving nodes use MSP430 microcontrollers [2] and CC2500 radio transceivers [2].

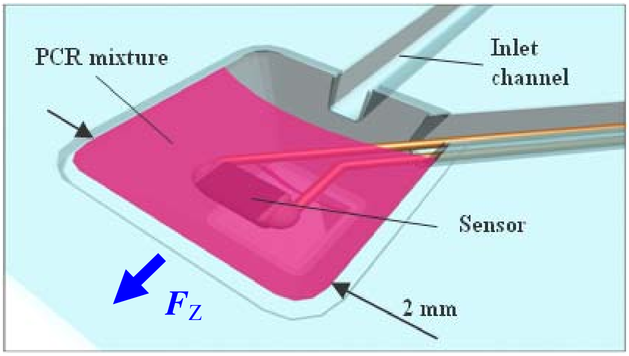

Figure 7: Cavity with 6 µl of reagent (red) and thermistor - F_z as centrifugal force exhibiting a flat liquid-air interface.

The power signal of 12 VAC is provided by the half-bridge self-oscillating MOSFET driver IR21531 [2] via a primary copper coil with ~60 mm diameter by inductive coupling at near-resonant frequency of ~90 kHz to a secondary copper coil of similar diameter fixed on the rotating carrier. Processed by the voltage rectifier LP2980 [2] a controlled voltage signal of 3.3 VDC finally powers both the rotating transmitting node and the sensor. Alternatively a tiny accumulator with a nominal voltage of 3.6 V could be used as a flexible energy source for the rotating elements to the advantage of design flexibility and system portability to any analyser available.

System controller

The wirelessly transmitted temperature data is processed by an embedded adaptive PID controller running on the MSP430 of the stationary board. Due to the non-linear behavior of the controlled system and 2 different actuators for heating by an IR source and cooling by air ventilation as well as the disk rotation a look-up table with setpoint temperature depending parameters for the PID control algorithms is implemented in a process controller running on the master PC. This process controller programmed in C# communicates via an UART interface with the temperature controller and enables thermocycling with free choice of temperature settings, holding times and cycle numbers (figure 8).

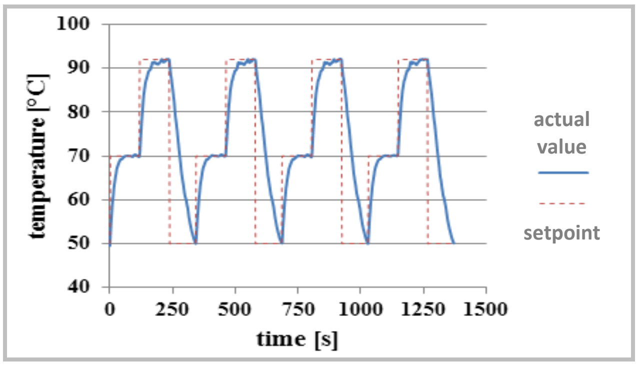

Figure 8: Thermocycling with free choice of temperature settings, holding times and cycle numbers by C# process controller communicating via UART with embedded controller.

EXPERIMENTAL RESULTS

We selected the IR ring heater of type Omega IR beamer 2D as its peak intensity hits an absorption maximum of water and a transmission maximum of the COC sealing film (figure 9).

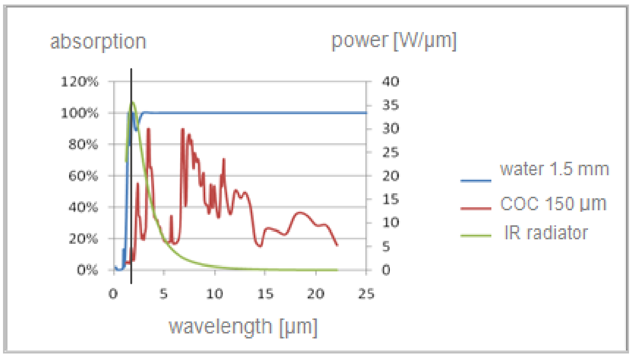

Figure 9: Absorption spectra of water and COC film in comparison to emission spectrum of IR radiator Omega IR beamer 2D.

At the IR radiators peak wavelength of 1940 nm approximately 15 % of the radiation energy is absorbed by the 150 µm COC sealing film, the rest by the aqueous reagent within the first 1.5 mm of depth (figure 10).

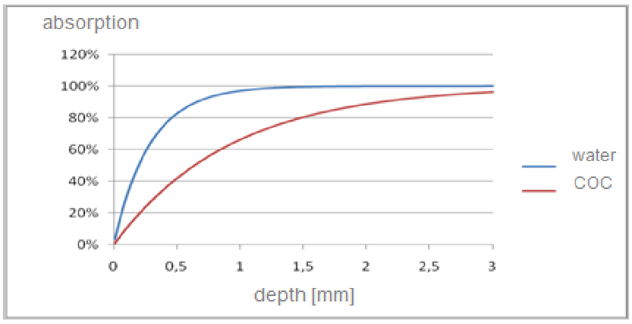

Figure 10: Absorption rates of water and COC for a wavelength of 1940 nm at various depth.

Simulations show that thermal conduction from the sealing and the cavity walls to the reagent contribute little to the heating process. Average heating rates of up to 4 K/s for step responses from room temperature to 50, 70 and 92 °C could be achieved while having low overshoots and stable temperatures (figure 11).

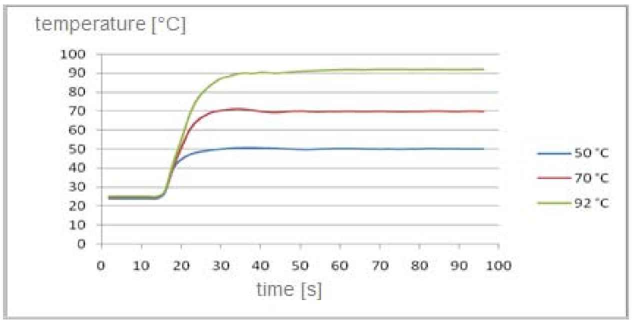

Figure 11: Temperature step responses of closed-loop PID controlled system measured in reagent cavity.

Cooling is controllable by both air ventilation and the disk rotation frequency. Increasing the rotation frequency from 3 Hz to 30 Hz reduces the thermal resistance by >60% (figure 12).

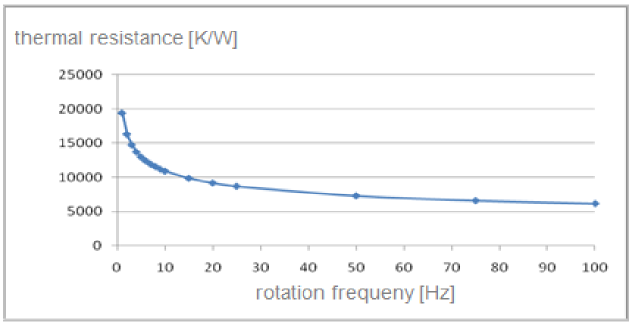

Figure 12: Thermal resistance of COC polymer film disk cavities at different rotation frequencies.

CONCLUSION

IR radiation enables to directly heat aqueous reagents in cavities of microfluidic polymer film disks with high temperature gradients. It requires the optimization of the energy flow by suitable reflectors, high transmission rates of disk materials and high absorption rates of the reagent at the radiators peak frequencies. Cooling by air convection can be simulated by analogous electrical models of the heat transfer and thus optimized. Thermocycling is flexibly mastered by a programmable process controller realized in C#. Adaptive PID algorithms embedded on a MSP430 perform well as enhanced temperature controllers. SMD thermistors are fully suitable for highly sensitive and robust temperature measurements in cavities of centrifugal microfluidic disks. The thermal capacity of the thermistor in comparison to the reagent should be very small in order to avoid any significant influence of the tempering process. A volume ratio of ~ 1:100 (54 nl (thermistor) / 6 µl (reagent)) in our setup fully meets this demand. Any variation of the ambient temperature between 25 °C and 70 °C of the rotating electronic components is not affecting the reproducibility of the temperature measurement. Thus this total system has the potential to reduce PCR cycling times significantly.

ACKNOWLEDGEMENTS

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CONTACT

* J. Burger, juergen.burger@imtek.uni-freiburg.de