IR thermocycler for centrifugal microfluidic platform with direct on-disk wireless temperature measurement system

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ABSTRACT

The direct on-disk wireless temperature measurement system [1,2] presented at µTAS 2010 was further improved in its robustness. We apply it to an IR thermocycler as part of a centrifugal microfluidic analyzer for polymerase chain reactions (PCR). This IR thermocycler allows the very efficient direct heating of aqueous liquids in microfluidic cavities by an IR radiation source. The efficiency factor of this IR heating system depends on several parameters. First there is the efficiency of the IR radiator considering the transformation of electrical energy into radiation energy. This radiation energy needs to be focused by a reflector to the center of the cavity. Both, the reflectors shape and the quality of the reflecting layer affect the efficiency. On the way to the center of the cavity the radiation energy will be diminished by absorption in the surrounding air/humidity and especially in the cavity lid of the microfluidic disk. The transmission spectrum of the lid material and its thickness is of significant impact. We chose a COC polymer film with a thickness of 150 µm. At a peak frequency of the IR radiator of ~2 µm approximately 85 % of the incoming radiation energy passes the lid and is absorbed within the first 1.5 mm depth of liquid in the cavity. As we perform the thermocycling for a PCR, after heating to the denaturation temperature of ~ 92 °C we need to cool down rapidly to the primer annealing temperature of ~ 55 °C. Cooling is realized by 3 ventilators venting air of room temperature into the disk chamber. Due to the air flow itself and an additional rotation of the centrifugal microfluidic disk the PCR reagents in the cavities are cooled by forced air convection. Simulation studies based upon analogous electrical models enable to optimize the disk geometry and the optical path. Both the IR heater and the ventilators are controlled by the digital PID controller HAPRO 0135 [3]. The sampling frequency is set to 2 Hz. It could be further increased up to a maximum value being permitted by the wireless temperature data transmission system. As we are controlling a significantly non-linear process the controller parameters need to be optimized for all temperatures relevant for the PCR thermocycling process. Such we get a dynamic system for both, the heating and the cooling process. Heating rates up to 5 K/s with our IR heater (100 W electrical power) could be achieved. Cooling rates of instantly 1.3 K/s at 20 Hz rotation frequency could be even further increased by higher rotation frequencies, faster air circulation, optimization of the controller parameters or an active air cooling unit.

Keywords: lab-on-a-chip, centrifugal microfluidics, PCR, Immunoassay, thermocycling, IR radiator, thermistor, wireless temperature measurement system

1. INTRODUCTION

Both immunoassays [4] as well as polymerase chain reactions (PCR) [5] require the tempering of the reagents. In microfluidic lab-on-a-chip (LoaC) systems [6] these reagents are generally kept in microreactors made of polymers with volumes of few microliters (figure 1). Cartridges with microfluidic control structures and cavities as microreactors could be both, injection-moulded as depicted in figure 1 or blow-moulded of foils [7]. Energy by any external source for heating or cooling the reagents has to pass the walls forming the cavity. The heat transport is affected by geometrical parameters of the cavity walls e.g. thickness and physical parameters e.g. heat conductance, transmission rate. The temperature of the reagent yielded by a certain amount of energy being transmitted into the reagent is further depending on the reagents volume and the heat capacity of its surrounding polymer cavity. The objective of having a robust system granting a reagent temperature accuracy of +/- 0,1 K despite fuzzy conditions as production tolerances of the cartridge or varying reagent volumes demands for both, a direct temperature measurement system and a precisely controllable energy source. State-of-the-art centrifugal analyzers do not apply direct temperature measurements [8]. They are model-based and control the temperature in the microreactor by measuring the temperature of circulating air. The temperature precision can only be as good as the model - any geometrical variations of the polymer cavity or its position on the disk, any production tolerances of the disk or any inaccuracy of the reagents volume in the microreactor diminish the temperature accuracy and hence potentially deteriorate the performance and especially the reproducibility of the assay or the PCR. Measuring the reagent temperature directly enables to use direct heating systems. Instead of transmitting the heat energy by convection from tempered air to the polymer cavity walls and thermal conductance through the polymer to the fluidic reagent the fluid in the microreactor can be heated electromagnetically. Heating by microwaves [9] could be considered however, we apply an IR radiator as an easy controllable energy source which can be handled without considerable safety restrictions. Prior to designing our system we consider the theoretical aspects of heat transfer by radiation, further the energy flow by conductance and convection. We develop a thermal network model for some elementary simulation studies [10].

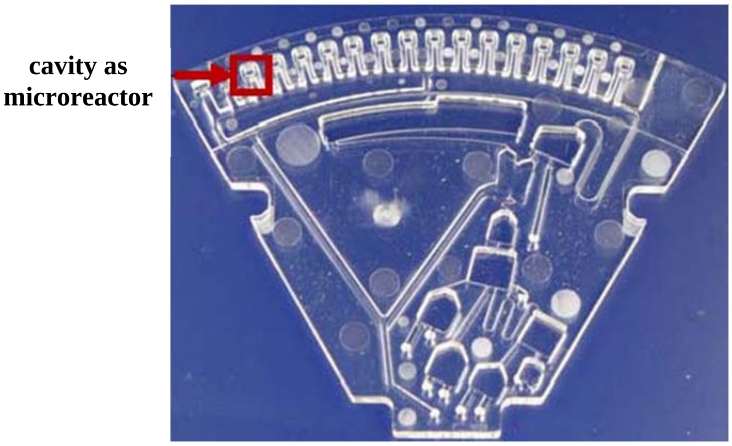


Figure 1: Injection-moulded microfluidic cartridge with 16 microreactors of COC polymer with cavity walls of 800 µm thickness (HSG-IMIT, Microfluidic Chipshop, Germany).

2. SYSTEM COMPOSITION

2.1 Temperature measurement system

The temperature measurement systems consists basically of rotating elements for data acquisition fixed on the cartridge carrier and stationary components for power supply and data processing (figure 2). A NTC (negative temperature coefficient) thermistor [1] used as temperature sensor is placed directly in one of the microreactor-cavities (figure 3).

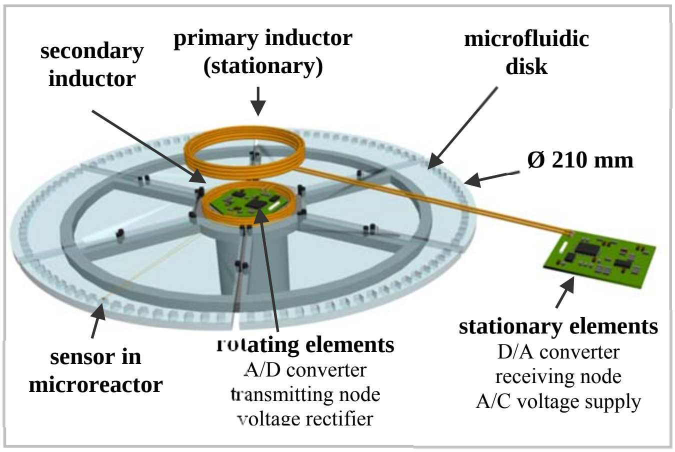


Figure 2: Wireless temperature measurement consisting of rotating and stationary elements.

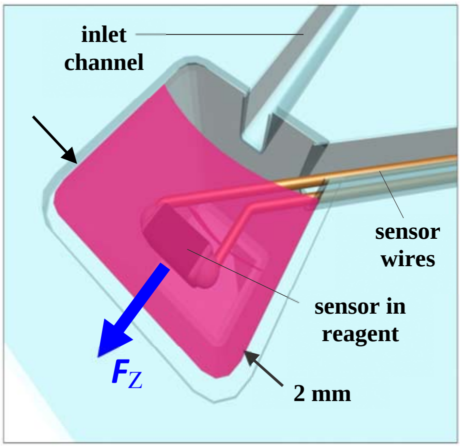


Figure 3: Microfluidic cavity with 6 µl of reagent (red) and sensor - F_z as centrifugal force exhibiting a flat liquid-air interface.

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 [1]. 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 [1] to a stationary receiving node at a data rate of 100 Hz. Both the transmitting and receiving nodes use MSP430 microcontrollers [1] and CC2500 radio transceivers [1]. Now the signal is D/A converted [1] and scaled to the standardized 0-10 V temperature controller interface [3]. The power signal of 12 VAC is provided by the half-bridge self-oscillating MOSFET driver IR21531 [1] 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 [1] a controlled voltage signal of 3.3 VDC finally powers both the rotating transmitting node and the sensor connected to the A/D converter. These modules are arranged in a centrifugal test rig (figure 4) enabling the direct temperature measurement of the fluidic reagent in the reaction chamber (figure 5).

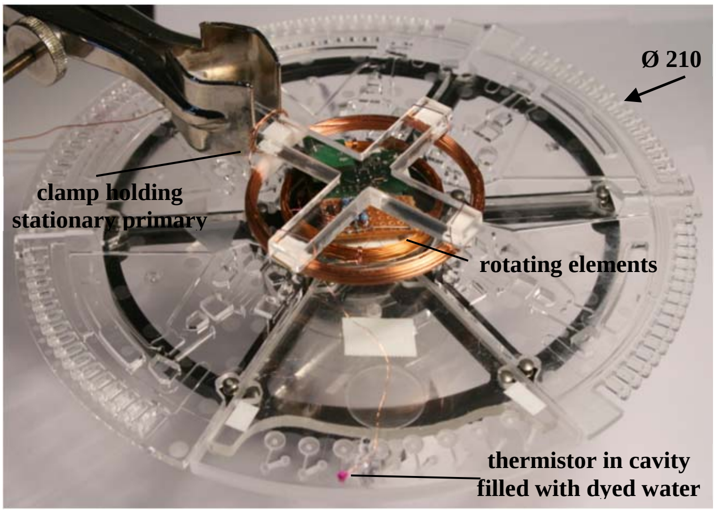


Figure 4: Setup with thermistor, A/D converter, transmitting node, primary and secondary coils.

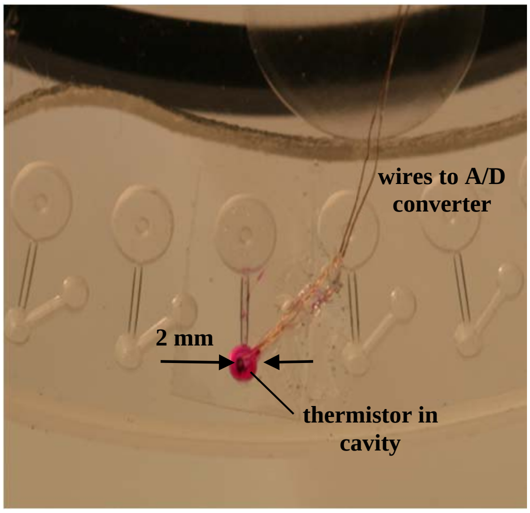


Figure 5: NTC thermistor fixed in a sealed PCR reaction chamber of a polymer film disk.

2.2 Tempering system

Designing an appropriate tempering system requires to consider the system parameters affecting the energy flow. As we are aiming to have a system being capable to perform an efficient thermocycling, we are analyzing the energy flows for cooling and heating the reagent in the polymer cavity. Cooling is realized by forced convection due to the cavity rotating in air of room temperature.

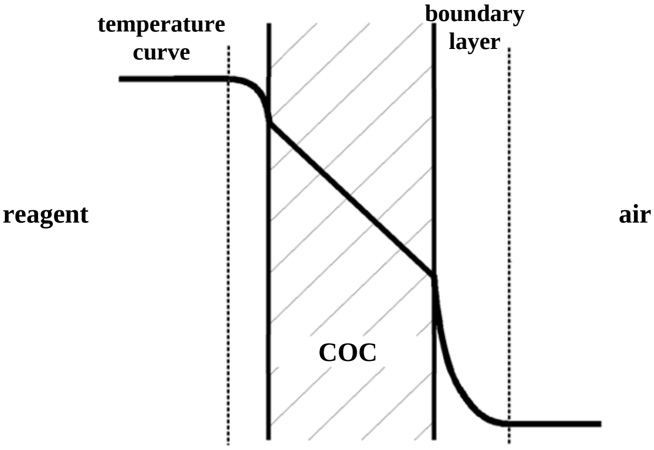


Figure 6: Heat energy transfer from reagent through polymer cavity wall to surrounding air by thermal conductance and convection.

The thermal boundary layers with their heat transfer coefficients α_{air} and $\alpha_{reagent}$ as well as the heat conductance coefficient λ *[W m^{-1} K^{-1}]* of the COC polymer and the polymers thickness *d [m]* determine the heat transfer. The total conductance *k [W m^{-2}K^{-1}]* is calculated by the formula 1 as parallel circuit of all single conductances:

$$k = \frac{1}{1/\alpha_{air} + d/\lambda + 1/\alpha_{reagent}} \tag{1}$$

This total conductance *k* can now be used to describe the heat flow from the reagent via the COC to air [10]. Averaging the relevant cross sections to *A [m²]* and using *ΔT [K]* as the difference between the temperatures of the reagent and the air we get the thermal energy transferred by the integral over time of their product. As both α_{air} and $\alpha_{reagent}$ are functions of the temperature the variable *k* is temperature dependent.

$$Q_k = k * A * \Delta T * \Delta t \tag{2}$$

We now perform simulation studies based upon an analogous electrical model as R-C network [10] with LTspice [11]. Resistors are depicting the inverse of the thermal conductance or convection, capacitors represent thermal energy stores. This system allows simulating the heat flow (current) and the resulting temperatures (voltage) at any node as a function of temperature and time.

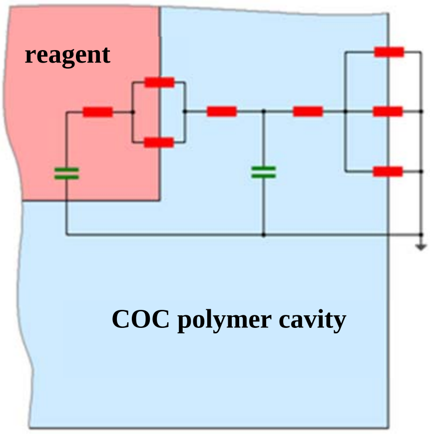


Figure 7: Analogous electrical model of heat energy transfer from reagent through polymer cavity wall to surrounding air by heat conductance and convection.

Heating is realized by IR radiation. In a first setup we apply an IR lamp [12] with a gold plated parabolic reflector as 100 W energy source (figure 8).

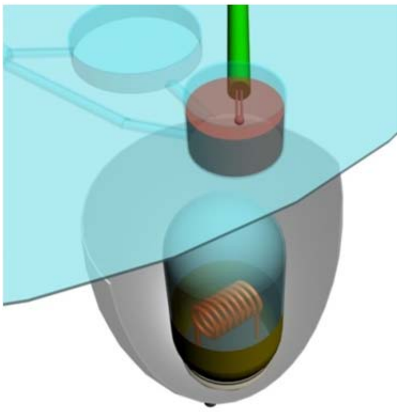


Figure 8: IR radiator with parabolic reflector as heating energy source.

3. EXPERIMENTAL RESULTS

3.1 Temperature measurement and process control

The voltage temperature characteristic of the thermistor (figure 9) reveals a slightly non-linear behavior but is linearized by a look-up table for the standardized 0-10 V controller interface. The wireless data transmission has proven to be resistant to frequencies of rotation up to 30 Hz (figure 10). Average reception rates of 85 % combined with a data transfer algorithm repeating transfers up to 6 times at 2.45 GHz ensure a total reception rate of far more than 99 %.

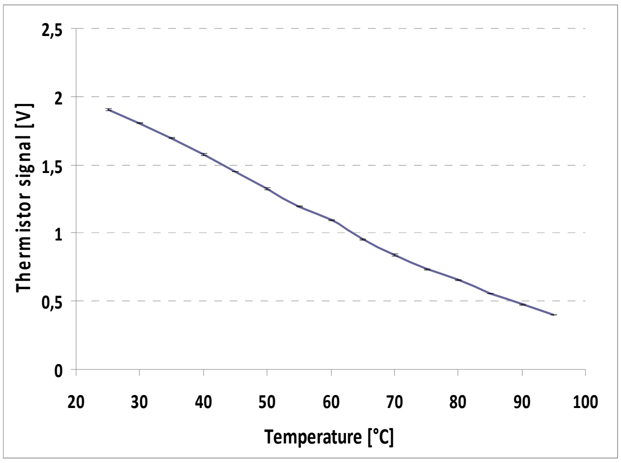


Fig. 9: Voltage temperature characteristic of thermistor.

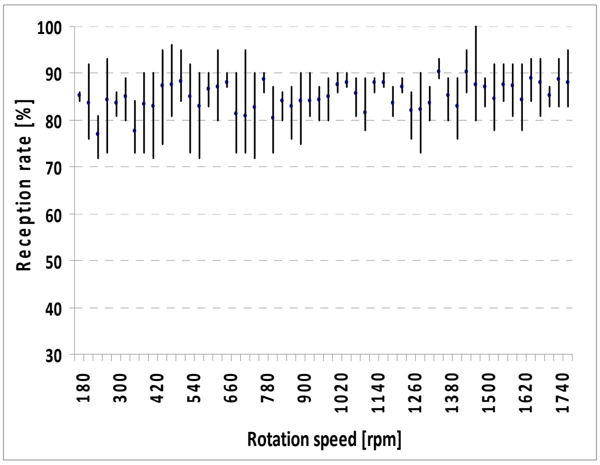


Figure 10: Wireless data transmission during rotation.

The step response of the total temperature measurement system from thermistor to wirelessly transmitted and again A/D converted signal as controller input reveals a delay of approximately 9.6 ms (figure 11). It is small in comparison to the response time of the heating system. Complete process control by a C# programmed PC user interface enables thermocycling with a free choice of temperature settings, holding times and cycle numbers (figure 12).

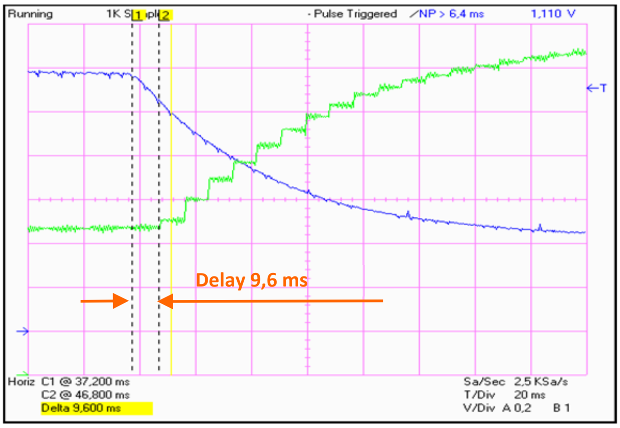


Figure 11: Step response of thermistor (blue) and transmitted D/A converted signal (green) after 50 K step.

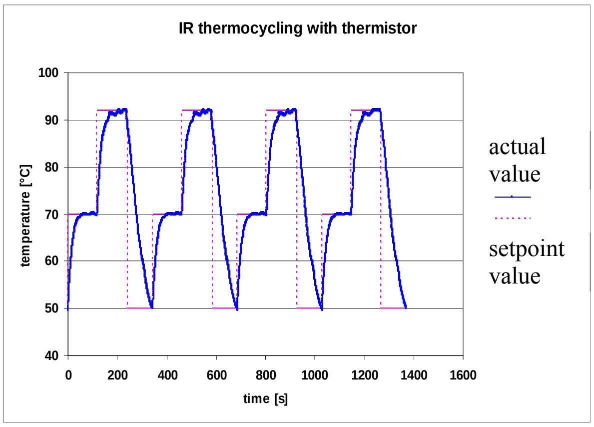


Figure 12: IR thermocycling with sensor in microreactor controlled by PID temperature controller.

The stability of the temperature measurement system has been analyzed by long term measurements of more than 2 hours. A PT100 element as calibrated reference sensor with a resolution of 0.005 K and an accuracy of 0.02 K depicts the temperature of oil (figure 13) in an open cavity of ~100 ml heated by the air incubator Stuart S119. Simultaneously the temperature of that oil is measured by the non-calibrated thermistor based system with a resolution of 0.1 K (figure 14). Further experiments have shown that even exposing the rotating electronical components of the temperature measurement system to ambient temperatures up to 70 °C are not affecting the stability of the temperature measurements.

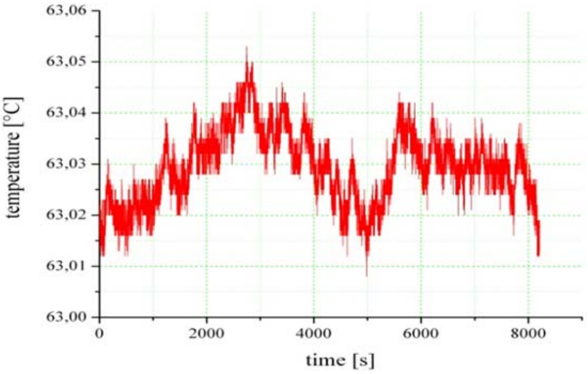


Figure 13: Long term experiment - temperature curve measured by calibrated PT100 element.

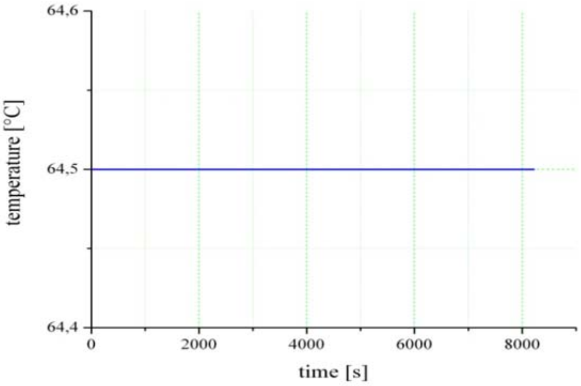


Figure 14: Long term experiment - temperature curve measured by non-calibrated thermistor.

3.2 Heating by IR radiation, cooling by forced convection

Direct heating of the reagent through the COC polymer cavity walls can be achieved by an IR radiator. Polymer films of 150 µm thickness lead to a transmission rate of ~85 % at ~2 µm wavelength (figure 15).

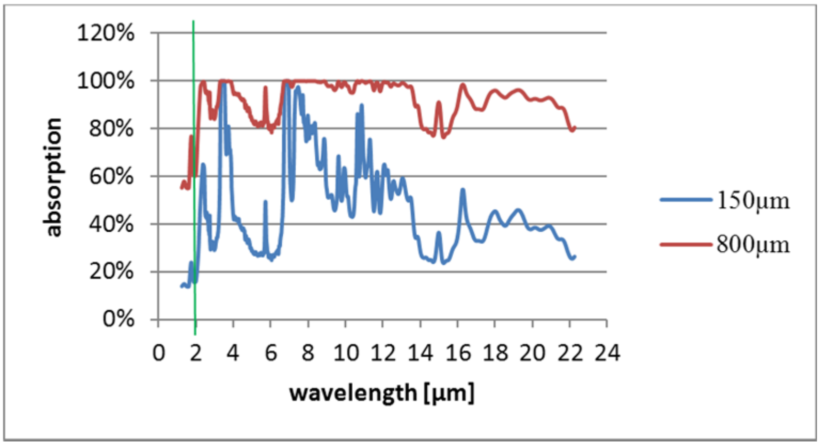
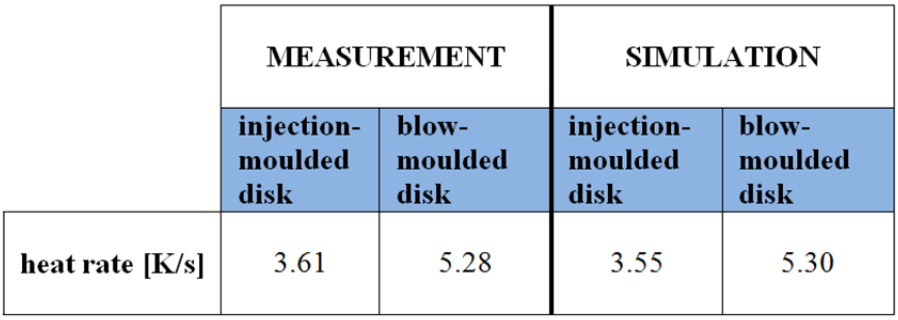


Figure 15: Absorption spectra for COC polymers of different thickness.

Heating rates of > 3 K/s can be reached for injection-moulded cavities with thickness of 800 µm, even more than 5 K/s for polymer film cavities of 150 µm thickness (figure 16 and 17). Both the simulation and the experiments lead to very similar results (figure 16).



	MEASUREMENT		SIMULATION	
	injection-moulded disk	**blow-moulded disk**	**injection-moulded disk**	**blow-moulded disk**
heat rate [K/s]	3.61	5.28	3.55	5.30

Figure 16: Heating rates in injection-moulded (800 µm thickness) / blow-moulded (150 µm thickness) cavities with 100 W IR lamp in comparison to simulations.

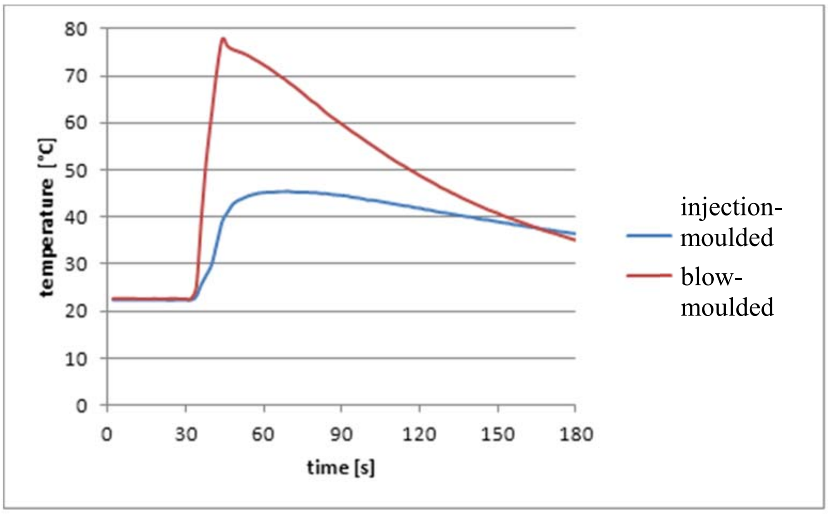


Figure 17: Heating and cooling gradients with 100 W IR lamp for injection-moulded / blow-moulded cavities.

The simulations of the cooling indicate a strong bias on the rotation frequency. Due to our restricted setup we could not perform sufficient experiments with rotation frequencies > 20 Hz yet. At 20 Hz a gradient of 1.3 K/s could be achieved.

4. CONCLUSION

SMD thermistors are fully suitable for highly sensitive and robust temperature measurements in cavities of centrifugal microfluidic disks. The vertical and horizontal position should consider the liquid-air interface under rotation. 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. Heating by IR radiation requires optimizing the energy flow by suitable reflectors, high transmission rates of housings 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 enhanced. Thermocycling is flexibly mastered by a programmable process controller realized in C#.

5. OUTLOOK

Having a robust and very dynamic temperature measurement system for centrifugal microfluidic disks with a resolution of 0.1 K now demands for increasing the resolution up to 0.04 K. Further we aim to improve the performance of the closed-loop temperature controller for both the IR radiator and the active cooling by fans. Embedded control by a MSP430 microcontroller would allow implementing adaptive PID algorithms and enables to increase the sampling rate up to the wireless data transmission rate. Thus by high but controllable gradients for heating and cooling the cycle times for PCR thermocycling could be further reduced.

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