CHARACTERIZATION OF AN INNOVATIVE POLYMERASE CHAIN REACTION DEVICE BASED ON BUOYANCY DRIVEN FLOW BY MEANS OF IR THERMOGRAPHY

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ABSTRACT

Polymerase Chain Reaction (PCR) plays a central role in the field of molecular biology. The miniaturization of PCR systems is promising as it potentially minimizes costly reagent consumption and time required for analysis. The present study focuses on the experimental characterization of micro-channels, having innovative and optimized shapes to obtain proper fluid actuation and DNA sample amplification within buoyancy driven flow PCR devices. In particular, this paper presents the first experimental results of surface temperature investigation by IR thermography. The IR thermography proved to be a critical tool to optimize the PCR device. In fact, the thermographic investigation allowed an optimal spatial arrangement of the two resistors on the chip to obtain a homogeneous temperature field on the two microchannel lateral walls. Moreover, thanks to the use of the thermocamera, it was also possible to find the correct voltage which granted the desired temperatures. Because of the small dimensions of the chip (20 mm × 20 mm), it was necessary to configure the optics in order to gain an adequate magnification.

INTRODUCTION

Since 1983 the Polymerase Chain Reaction (PCR) has been playing a central role in the field of molecular biology. It is a powerful tool for creating large numbers of copies of specific DNA fragments for applications like DNA fingerprinting, genomic cloning and genotyping for disease diagnosis [1]. The common devices used for PCR consist of metal blocks that are continually thermally cycled between three distinct temperatures for denaturation (90 - 94 °C), annealing (50 - 60 °C) and extension (72 °C). The advantage of these block thermal cyclers is that they can simultaneously amplify 96 - 384 samples placed in wells that are sandwiched between the thermal block and the cover plate. The disadvantage associated with this format is the poor thermal management which leads to limitations on the rate of amplification. Moreover the need for manually pipetting reagents into the wells limits the minimum volume of PCR reaction to about 0.5 ml [2]. In the last few years the limitations of benchtop thermal cyclers have been overcome thanks to the development of microfluidic PCR devices. These systems can generally be classified into two categories: the chamber type, where the DNA sample is placed and thermally cycled, and the continuous flow type, which consists of microchannels continuously looped through different temperature zones. The continuous flow type has the important advantage of being able to guarantee better thermal management (since each temperature required for the PCR is at equilibrium prior to target amplification); this positively affects amplification time reduction [3]. However continuous flow PCR devices require external pumping and have a fixed number of thermal cycles. In this light some alternative solutions have been recently developed, relying on a temperature-induced density difference in the presence of a body force to induce buoyancy driven flow [4] [5] [6]. This alternative method is easy to use and does not require expensive set-ups, but, to date, further efforts should be taken to optimize the thermofluid-dynamic field in the micro-channels. The main issue to address is the thermal cycling process control.

This project aims at experimentally characterizing by IR thermography an innovative-design, buoyancy-driven-flow PCR device. A preliminary computational study was first carried out, in order to get information about velocity and temperature fields. In the future, when the experimental characterization of devices filled with deionised water is performed, the computational analysis [7] will be a powerful tool to parametrically analyze the experimental results. This paper presents the experimental characterization of a PCR buoyancy-driven flow micro device carried out by means of IR-thermography which yields the surface temperature distribution on the device.

EXPERIMENTAL SETUP

The device was realized with two different materials, Polydimethylsiloxane (PDMS) and glass. In particular, a three dimensional microchannel with a square cross-section (W = 500 µm), and a total length of L = 5 mm, arranged in a closed loop, was engraved on a substrate of PDMS. In fact, the fabrication process of the devices is based on a PDMS replica molding technique: the negative of the channel was realized by draining fresh PDMS (weight ratio 10:1) on a mold, and heating at 100 °C for 30 min, to make the PDMS polymerizing.

The chip was sealed with a glass slide (Fig. 1), using a commercial innovative device (Corona Treater, Elechtro-technics Product, Illinois, USA), which makes use of a high potential electrode to ionize the surrounding air while creating a localized plasma. The plasma is able to activate the glass and polymer surfaces, granting so strong bound. After treating the surfaces, PDMS was pressed against the glass slide for about 60 s and kept in pressure for at least 24 hours.

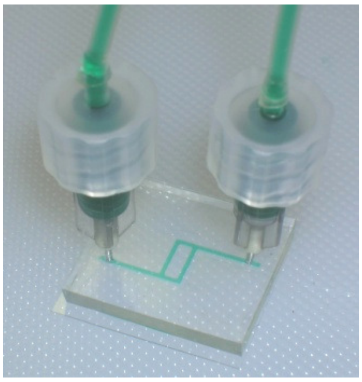


Figure 1. Picture of the designed device.

Therefore, two 100 Ω resistors were glued on the glass next to the long sides of the device using an appropriate conductive gel, in order to obtain a difference of temperatures between the two parts of the channel. Then, two needles were plugged in the PDMS and glued to provide inlet and outlet to the channel. The difference of temperature was established thanks to a power supplier.

A major problem was bubble formation inside the channel due to the high temperatures reached during the thermal cycle. In order to avoid this phenomenon, the pressure inside the device was set to a value of 2 atm to shift boiling point to higher temperatures, and thus preventing bubble formation.

The device surface temperature was measured by an IR-camera (Raytheon Radiance HS) with a 25-mK sensitivity, InSb Focal Plane Array detector, using the full resolution, i.e., 256×256 pixels. The IR-camera was calibrated by means of a point source blackbody with an accuracy of 0.1 K.

Some operative issues had to be addressed in order to allow thermographic characterization for this PDMS-glass device. First, PDMS emissivity was evaluated; second, a number of measurements were carried out to estimate the temperature difference between the glass slide and the PDMS sides, since resistors are glued on the glass side and the measurements of surface temperature by IR-camera are taken on the PDMS side. Finally, the IR-camera spatial resolution was estimated as the device has small dimensions (2 cm × 2 cm).

As concerns PDMS emissivity after surface was blackened by matt, this was estimated by heating the device with two halogen lamps and then comparing the temperature measured by the IR-camera with the values read by a thermoresistance PTR100 positively mounted on the surface to this end. As a result, we estimate the emissivity is equal to 0.96.

Finally, based on a simple thermal analysis, we evaluate the temperature difference between environment air and PDMS surface is of about 3 °C. This temperature difference is not negligible and hence it should be accounted for.

The last issue dealt with was related to the IR-camera resolution, which proved to be inadequate in order to accurately investigate the device area. The minimum camera focusing length is approximately 800 mm and, at this distance, the IR-camera frames a 140 mm × 140 mm area. Figure 2a shows the image recorded with the IR-camera during the first experiment whereas Figure 2b displays a magnification of the device area. It can be noticed the image resolution is very poor if compared with the overall dimensions of the micro-device; thus no characterization can be performed as it is not possible to distinguish any structures prior to execution of any statistics on the temperature field. Therefore, optics had to be reconfigured to gain an adequate magnification.

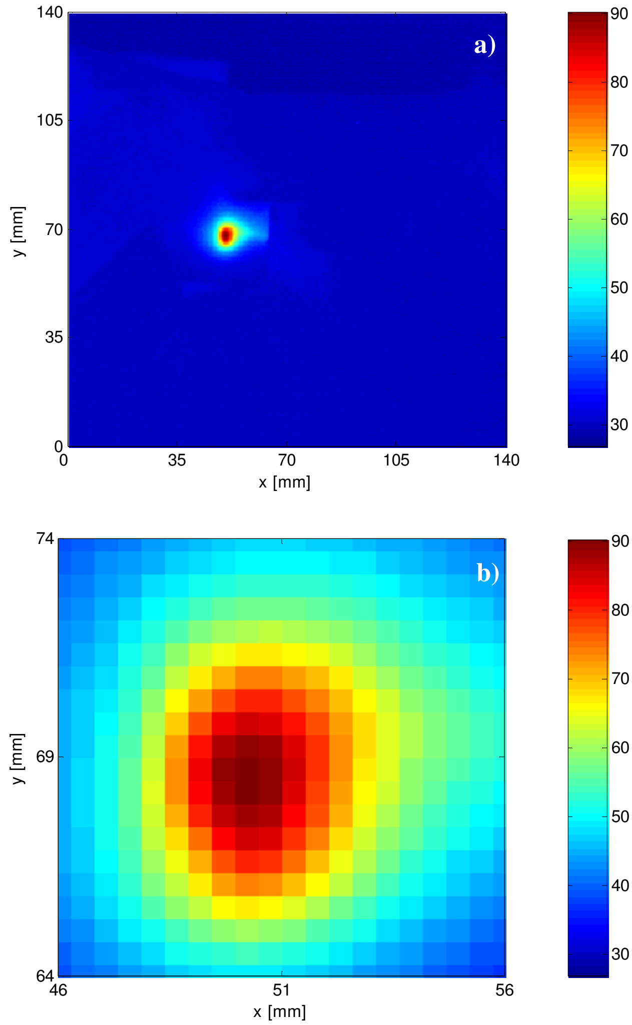


Figure 2. a) First acquisition by IR camera without bellow; b) Magnification to evaluate the temperature profile.

This optics rearrangement consisted in spacing out the objective from the camera by mounting an extension tube of 60 mm length. The draught was calculated using the equations of geometric optics in order to frame a 7 mm × 7 mm area which assures a fine resolution of 27.3 μm × 27.3 μm. Figure 4 shows an image obtained using the extension tube. In order to reduce the noise, all images of the thermal acquisitions are obtained averaging pixel by pixel 30 frames, recorded in a thermal equilibrium.

RESULTS AND DISCUSSION

The first experimental results obtained from the thermal analyses are here presented.

From the observation of the temperature field around the electric resistor, shown in Figure 3, it can be noticed that the resistor seem to be short compared with the channel length. Indeed, if we want to guarantee a uniform temperature over the whole channel length, an homogeneous field is needed. For this reason, we decided to place two resistors (w = 3.5 mm and l = 2.35 mm) one after the other to enlarge area at the same temperature, and to cover a wider portion of the channel branch.

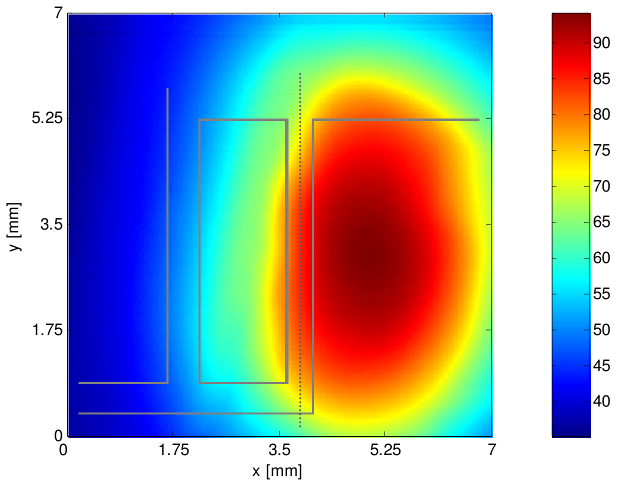


Figure 3. Temperature field [°C] on the micro-device with a resistance of 1.5 mm × 3.5 mm on the right branch of the channel, heated at a voltage V = 2.1V.

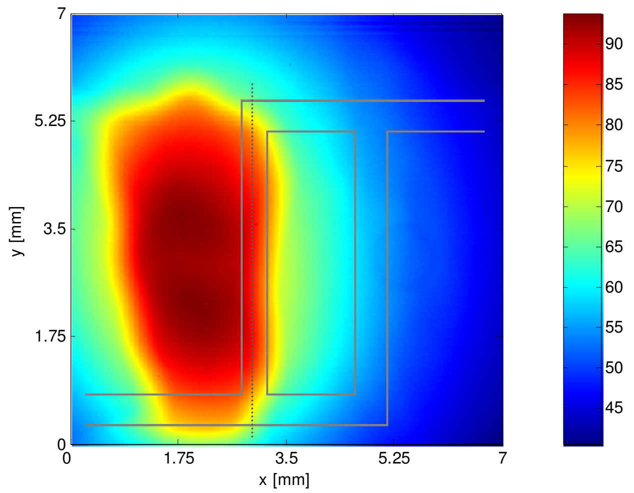


Figure 4. Temperature field [°C] on the micro-device with two resistors in series of 1.35 mm × 2.35 mm on the left branch of the channel supplied with a voltage V = 3.3V.

Figure 4 depicts a thermographic image where a more homogeneous temperature field is attained by using this configuration.

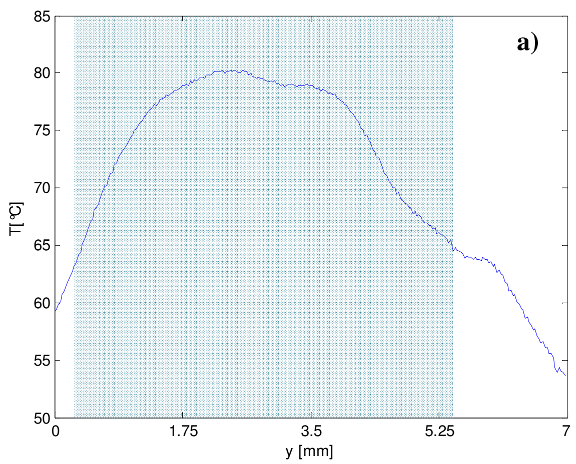
Figure 5 shows the temperature profiles in y-direction, derived from the temperature maps displayed in Figures 3 and 4 for x = 3.85 mm and x = 3 mm, respectively. If the 3.5 mm long, channel central-portion is considered, the mean temperature value is T = 77.91 °C (± 2.21 °C) for the device with a 1.5 mm × 3.5 mm resistor, whereas T = 78.04 °C (± 1.03 °C) for the device with two 1.35 mm × 2.35 mm resistors. If the entire length of the channel is taken into account, the value for the device with the two resistors becomes T = 75.85 °C (± 4 °C). This result is mainly due to the total resistor length that is shorter than the channel branch.

As a major indication from these images, the resistor should be further lengthened to guarantee a more uniform temperature along the whole channel length.

Regarding the temperature difference between the two vertical device branches, the temperature distribution is shown in Figure 6a for a device with two 1.35 mm × 2.35 mm resistors mounted one after the ather, and both supplied with a tension of 2 V.

One can easily notice quite different aspects of the temperature map in the two resistor areas. While a homogeneous field is obtained on the left side, on the opposite side the profiles are very irregular. This may be caused by a not enough accurate resistor positioning, as well as to excessive amount of conductive gel which creates a larger layer between resistor and glass slide.

Furthermore, Figure 6b displays (solid blue line) the temperature distribution along the horizontal grey line drawn in Figure 6a. The presence of two peaks corresponding to the resistors can be observed. In the same graph, the temperature trend, averaged on the central portion of the channel (dashed line), was plotted; the root mean square value between the two lines is σ = 0.81 °C. This value could be used as an indicator of temperatures homogeneity along the x-coordinate at different value of y.



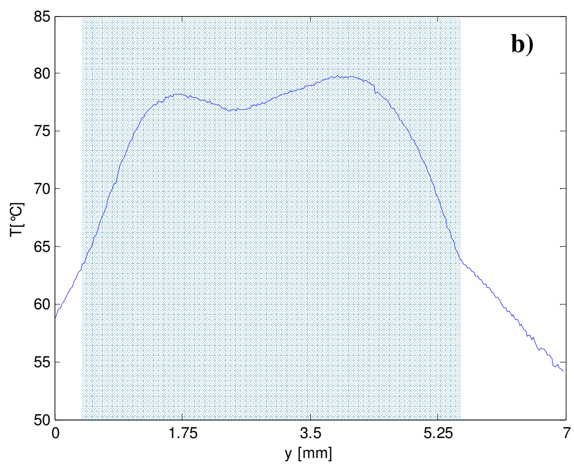


Figure 5. Temperature profiles extrapolated from the temperature maps in Figures 3 and 4 in correspondence to the midlines of the vertical channel branch: a) for the device with a 1.5 mm × 3.5 mm resistance; b) for the device with a series of two 1.35 mm × 2.35 mm resistors.

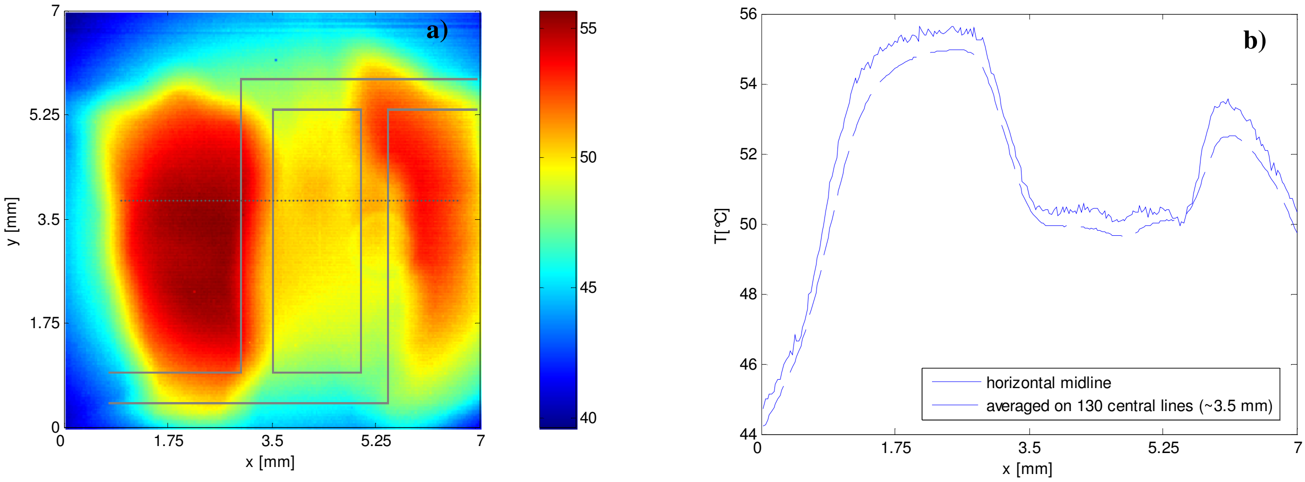


Figure 6. a) Distribution of temperatures for a device with 1.35 mm × 2.35 mm resistors in series, both supplied with a voltage V = 2 V; b) Temperature distribution along the horizontal grey line drawn in Figure 6a.

CONCLUSIONS

In the present study we considered a closed loop able to cycle a DNA sample between the annealing and denaturation phases. After cycling, the flow may be stopped and the extension phase may be achieved by setting both heaters at 72 °C. This system will allow for a DNA amplification of 3 μl of DNA sample solution in approximately 8 s.

An experimental characterization with IR-thermography was carried out to evaluate whether the temperature distribution along the vertical channel branchesis was satisfyingly uniform or not, that is a mandatory condition to assure an optimal thermal cycle of the sample inside the device.

This technique proved to be an effective tool in order to evaluate the distribution of temperature on the device surface. With the use of an extension tube mounted between the camera and its objective, the temperature field presents can be analyzed with a satisfying spatial resolution. This allowed us to get some indications concerning the length and the positioning of the resistors. It was also possible to check the correct placement of the resistors in terms of parallelism with respect to the lateral channel branches. Future developments will consider a number of tests with devices having longer resistors.

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NOMENCLATURE

Symbol	Quantity	SI Unit
V	Voltage	V
T	Temperature	°C

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