CENTRIFUGO-THERMOPNEUMATIC LIQUID ACTUATION FOR MICROFLUIDIC GENOTYPING OF NUCLEIC ACIDS

M. Focke[1], O. Strohmeier[1], P. Reith[1], G. Roth[1,2,3], D. Mark[2], R. Zengerle[1,2,3] and F. von Stetten*[1,2]

[1] Laboratory for MEMS Applications, Department of Microsystems Engineering – IMTEK, University of Freiburg, Germany;

[2] HSG-IMIT, Villingen-Schwenningen, Germany;

[3] BIOSS - Centre for Biological Signalling Studies, University of Freiburg, Germany

ABSTRACT

We report a novel principle to actuate liquids based on centrifugo-thermopneumatic effects. This approach enables us to reliably realize temperature-controlled valves and aliquoting structures on the centrifugal microfluidic platform. We present special geometries that force liquids to generate gas entrapments when exposed to a centrifugal field. The gas entrapments expand (or contract) when heated (or cooled) thus displacing the liquid volumes. Successful implementation of this principle is demonstrated by a microfluidic chip for automated real-time PCR based genotyping of nucleic acids.

KEYWORDS: Centrifugal microfluidics, Liquid actuation, Thermal pumping, PCR

INTRODUCTION

Development of lab-on-a-chip systems is driven by the great demand for automated and integrated solutions in the market of life science and molecular diagnostics. A particular challenge is the establishment of such microfluidic systems in conventional lab environments. One solution to this task is to automate standard laboratory devices by integration of microfluidic chips. Here we report on successful implementation of a high-impact application in a completely unmodified commercial thermocycler (Rotor-Gene, QIAGEN GmbH, Germany): We demonstrate a microfluidic chip for automated genotyping of nucleic acids that is based on passive temperature-controlled valves and aliquoting structures on the centrifugal microfluidic platform. Liquids are actuated by so-called centrifugo-thermopneumatic effects: As gas entrapments are generated during rotation, they can be expanded (or contracted) when heated (or cooled) during thermocycling. This is used to displace liquid volumes.

Compared to recent publications [1,2], the new actuation principle not only allows to control liquids without the need for changing rotational speeds, it also enables to run analytical applications in unmodified commercial centrifugal thermocyclers.

FABRICATION AND WORKING PRINCIPLE

The chip design is shown in Fig. 1. Chips are based on polymer foils and are microthermoformed as described in [1]. The chip is filled with 165 µl of sample liquid and the inlet hole is sealed with an adhesive lid. Then the chip is attached to a carrier which is placed in a Rotor-Gene rotary thermocycler (Fig. 2). When starting a run, the chip rotates at 400 rpm generating a gas entrapment in the inlet reservoir. When the chip is heated to a temperature of 80 °C, the entrapped gas expands and pushes the liquid through the overflow siphon (Fig. 3b). Thus the liquid is released into the distribution channel with 8 finger-like metering chambers with 20 µl each (Fig. 3c). The 20-µl aliquots do not flow into the adjacent reaction wells due to a pneumatic counter-pressure [3] (Fig. 3d). Decreasing the temperature results in contraction of the gas volumes in the reaction wells, and aliquots virtually become sucked into the wells (Fig. 3e). This process is additionally supported by continued centrifugal force at constant 400 rpm.

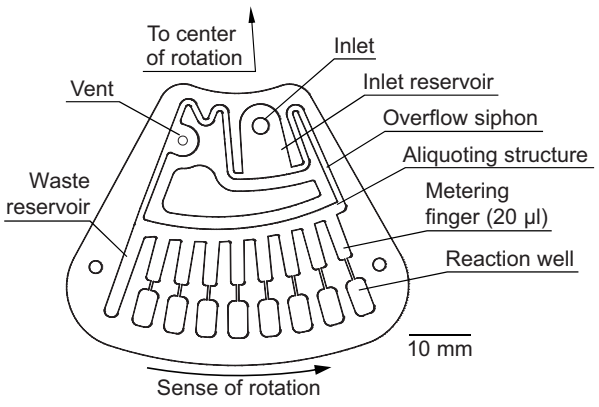

Figure 1: Schematic design of the microfluidic chip.

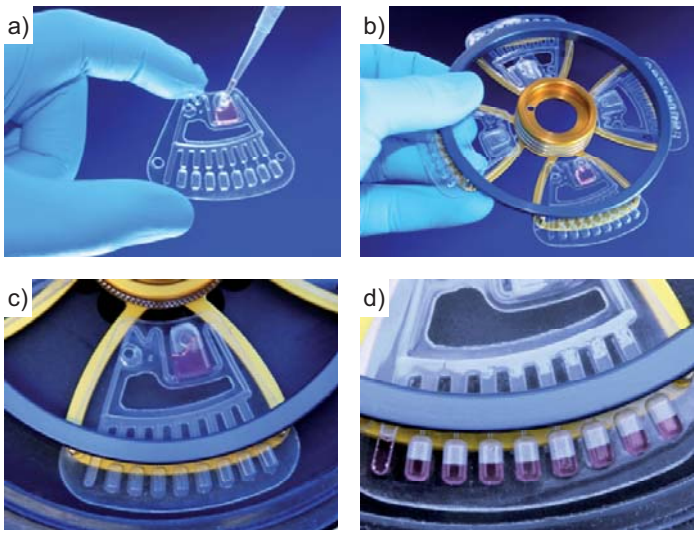

Figure 2: Process flow. (a) Loading of a chip with 165 µl of a sample (here dyed water). (b) Loading of the carrier with four chips. (c) Carrier in Rotor-Gene. (d) Aliquoted liquid sample in reaction wells after centrifugo-thermopneumatic aliquoting.

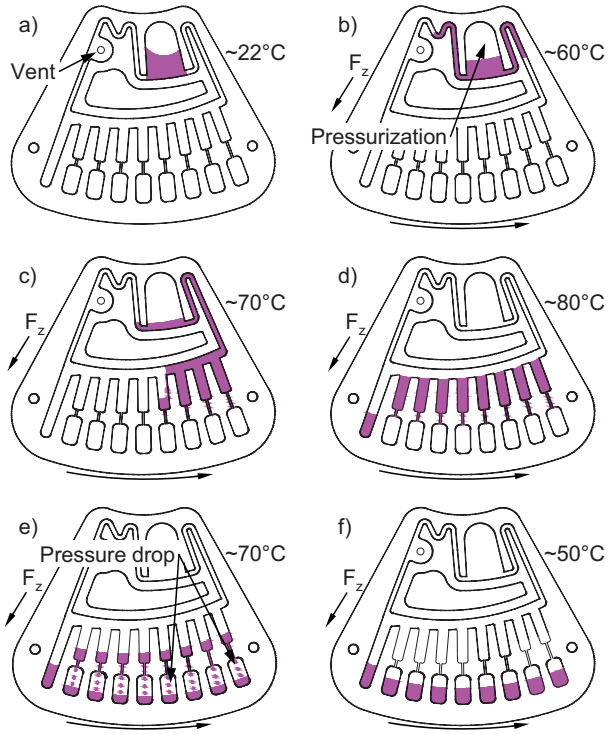

Figure 3: Microfluidic processflow. (a) Sample liquid is filled into the inlet reservoir and the inlet hole is closed. (b) The rotational protocol starts and generates a centrifugal force F_z. Simultaneous heating induces a pressurization of the inlet reservoir acting on the liquid. (c) The overflow siphon releases the liquid into the aliquoting structure. Metering fingers become filled. A counter pressure in the reaction wells prevents filling of the wells as described in [3]. (d) All metering fingers are filled. Gas volumes with a temperature of 80°C are encapsulated in the reaction wells. (e) A temperature decrease causes a pressure drop in the reaction wells thus sucking in the liquid aliquots. (f) The reaction wells are filled with equally distributed aliquots at the end of the flow cascade, and DNA amplification can start.

RESULTS

Specific primers and probes of (a) *Corynebacterium glutamicum* and (b) *Escherichia coli* (both labeled with different fluorescent probes) were pre-stored in dry form in alternating reaction wells of a chip. A liquid sample containing gDNA of *E. coli* and *C. glutamicum* was processed as shown in Fig. 3. QuantiFastProbe (QIAGEN GmbH, Germany) was used as PCR mastermix. After centrifugo-thermopneumatic valving and aliquoting, a real-time PCR run was started directly. The amplification plots (Fig. 4) show that all dried reagents were properly rehydrated and DNA samples were correctly amplified in their respective reaction wells. This proves feasibility of method and application.

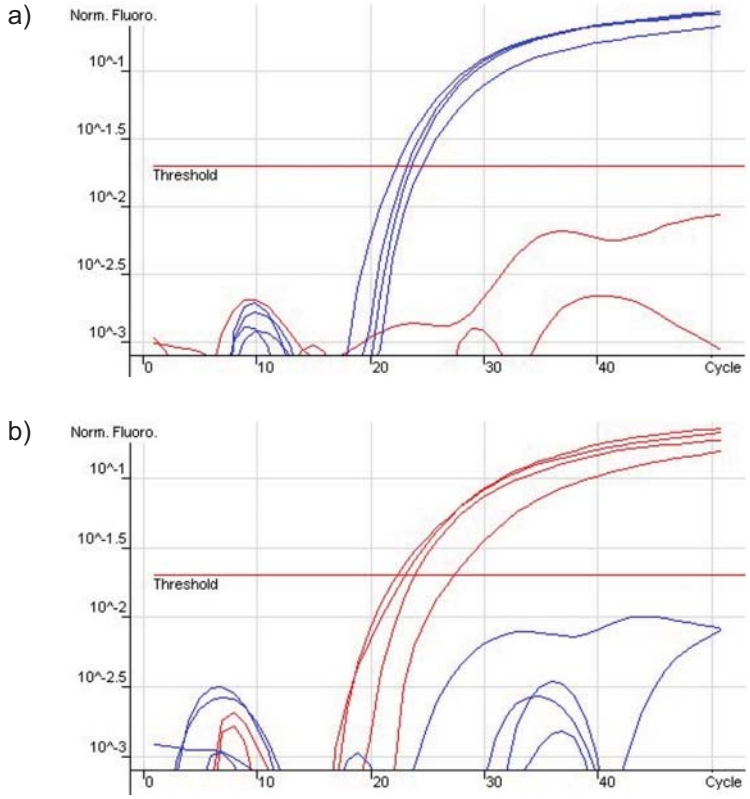

Figure 4: Genotyping assay performed in the microfluidic chip. The sample contained a mixture of different gDNA of (a) C. glutamicum and (b) E. coli which were each labeled with different fluorescent probes (FAM and JOE, respectively). The plots show correct and cross-contamination free amplification of the sample in the respective reaction wells.

CONCLUSION

Centrifugo-thermopneumatic actuation increases the degree of freedom for design of microfluidic chips and even enables microfluidic systems for complex multi-parameter screening assays. Processing of microfluidic chips in unmodified standard lab instruments adds to high convenience and promises some attractive market potential.

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CONTACT

* Dr. Felix von Stetten, phone: +49-761-203-7393; vstetten@imtek.de