PARALLEL DNA AMPLIFICATION USING LOCALIZED MICROWAVE HEATING IN STANDARD-MICROTUBES

W. Hilber[1*], I. Tiemann-Boege[2], C. Diskus[1], T. Lederer[1], B. Jakoby[1] and J. Boulanger[3]
[1]Institute for Microelectronics and Microsensors, Johannes Kepler University Linz, AUSTRIA
[2]Institute of Biophysics, Johannes Kepler University Linz, AUSTRIA
[3]Johann Radon Institute for Computational and Applied Mathematics, Academy of Sciences, AUSTRIA

ABSTRACT

This work presents the utilization of an open-ended semirigid coaxial cable as a miniaturized microwave heating source used in standard 0.2ml microtubes for PCR amplification of single DNA-molecules. With our set-up we achieved the successful amplification of single molecules in a microscopic format. Specifically, single molecules are compartmentalized in microscopic reaction volumes formed by an emulsion. The amplification product is captured on microscopic paramagnetic beads that can then be analyzed under a microscope. Using this small PCR format, we have proof of principle that our device can be used in miniature PCR and is suitable for lab-on-a-chip developments. Moreover, the ease of fabrication and the utilization of standard power RF-components make this set-up suitable for mobile developments also in other technologies.

KEYWORDS: microfluidics, RF-electronics, DNA-amplification, mobile lab-on-a-chip, microscopic PCR

INTRODUCTION

Only recently it has been shown that dielectric heating of water can in principle be used to drive flows in microchannels [1]. Almost coincidentally it has been suggested to utilize localized microwave heating in microwells for DNA amplification [2]. In these studies a microfluidic well integrated with a microwave transmission line in microstrip geometry, realized in MEMS technology, was applied to heat a fluid sample dielectrically. However, the reported maximum temperature was 72°C and the experimental proof of DNA amplification was unperformed.

In this work we overcome temperature limitations by using standard power RF-components, originally developed for mobile wireless communication applications. Microwave fields with an incident power up to 34dBm (~2.5W) and with frequencies in the range between 1 and 7GHz were applied to fluid samples. We show that the open end of a miniature coaxial cable can inject microwave fields into the fluid of a microtube leading to localized heat induction with continuous flow conditions and an associated temperature field. By means of material choice and device geometry design the necessary temperature zones for natural convection PCR were obtained.

THEORY AND SIMULATION

Figure 1 shows a sketch and a photograph of the experimental setup. The semirigid coaxial cable with an outer diameter of 1.1mm was inserted together with a small thermocouple (wire diameter 25μm) responsible for the temperature control through an access hole in the cap into the 0.2ml microtube (shown in Figure 1).
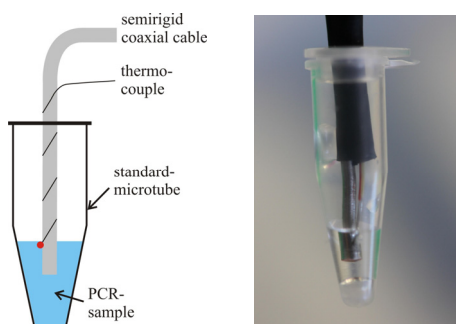

Figure 1: Sketch (left) and picture (right) of the experimental setup, depicting the microtube, the inserted semirigid coaxial cable, and a small thermocouple near the liquid surface for temperature control. The PCR reaction is performed with the thermal convection flow formed between the heat source in the center and the colder periphery.

Energized by a microwave generator, the open end of the coaxial line leads to localized dielectric and, depending on applied frequency and ionic strength of the sample, also resistive heating of the fluid. Gradients in temperature T produce spatial variations in mass density ρ and permittivity ε. However, the geometric dimensions of the device well above 100μm ensure dominance [1] of buoyancy forces

$$\boldsymbol{f_g} = \Delta\rho \cdot \boldsymbol{g} = \left(\frac{d\rho}{dT}\right) \cdot \Delta T \cdot \boldsymbol{g} \tag{1}$$

over dielectric forces

$$\boldsymbol{f_E} = -\frac{1}{2} \cdot E^2 \cdot \boldsymbol{\nabla}\epsilon , \tag{2}$$

which lead to convective fluid flow in the microtube. FEM-simulations of the temperature distribution and the resulting flow field in the microtube were performed with COMSOL MULTIPHYSICS 3.4, using the MEMS-module. Figure 2 shows an example of the simulated temperature distribution and resulting flow fields determined by both the localized inward heat flux and the contact area of the outer metal sheath of the coaxial cable with the fluid, acting as a heat sink.
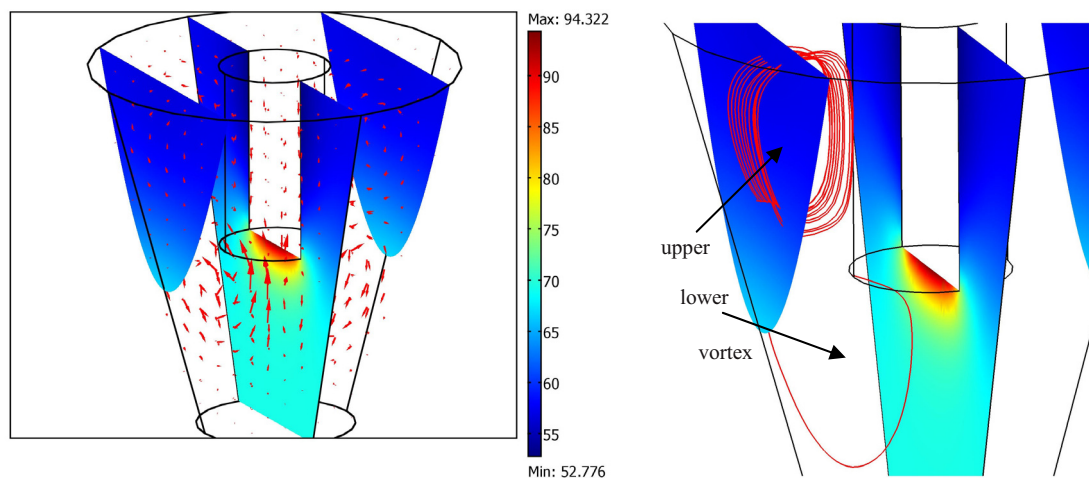

Figure 2: (left) Simulated temperature distribution in °C (color bar) and the resulting flow fields (arrows) in the microtube filled with 25µl 0.9% NaCl-solution for an incident microwave power of 24dBm at 2.1GHz. The outer metal sheath of the semirigid coaxial cable immersed into the liquid acts as a heat sink, yielding the necessary temperature zones for natural convection PCR in the microtube. (right) Simulated particle trajectory in the flow field illustrates the interaction of two vortices.

Due to geometry and the thermal boundary conditions two connected vortices are formed. First, fluid circulates directly underneath the heat source. Next, a weaker component circulates the fluid in a zone with decreased temperature in the upper part of the microtube. Particle tracing simulations show that the fluid circulates several times in the upper vortex, before it is captured by the lower vortex, which brings the fluid directly in front of the heat source (see right panel in Figure 2). There the fluid is locally heated, goes up due to the dominance of buoyancy forces, until it escapes to the upper vortex again. The steady state convection shuttles the reaction molecules regularly between the hot center in front of the open end of the coaxial cable in the center of the microtube and the colder periphery. DNA is exponentially amplified as it melts in the center and is duplicated by proteins in the periphery.

EXPERIMENTAL RESULTS

As a model fluid for device characterization a 0.9% NaCl-solution was chosen. Figure 4 shows the measured reflection coefficient S_{11} of the microwave signal, from which the fraction of dissipated microwave power can be obtained, as well as the measured and simulated frequency dependence of the heating efficiency for a fixed incident microwave power of 17dBm.
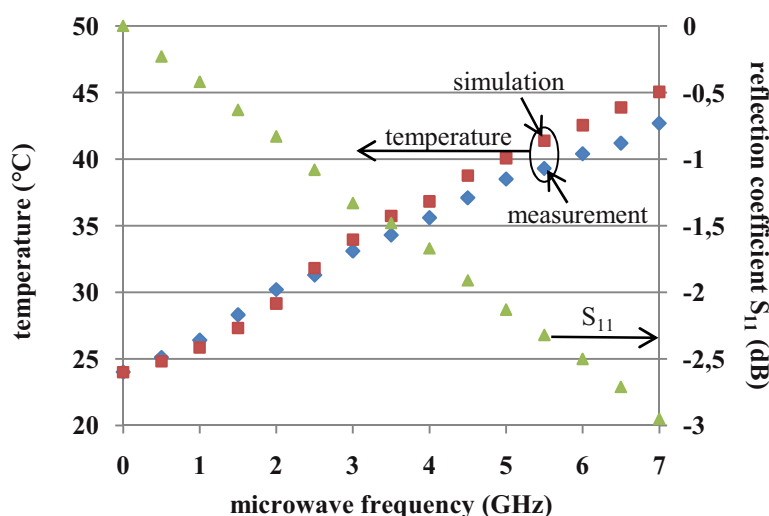

Figure 4: Heating efficiency in a 0.9% NaCl-solution: Measured temperature rise near the liquid surface (see position of thermocouple in Fig. 1) as a function of microwave frequency with constant incident power of 17dBm, as well as the simulated values based on the measured reflection coefficient S_{11} of the microwave signal.

As expected, the reflection coefficient S_{11} of the microwave signal decreases with increasing frequency, which corresponds to increasing absorption efficiency. The fraction of dissipated power P_{diss} can be obtained according to

$$P_{diss} = P_{1+} - P_{1-} = P_{1+} \cdot \left(1 - 10^{\frac{S_{11}}{dB}/10} \right), \tag{3}$$

where P_{1+} and P_{1-} denote the incident and reflected microwave power, respectively. For the FEM-simulations it is assumed that the dissipated microwave power P_{diss} is entirely converted into heat, either by the actual dielectric microwave heating, or to some extent, depending on ionic strength of the solution and frequency of the applied microwave signal, also resistive heating. This assumption is well supported by the congruence of simulation and experiment, as shown in Figure 4. Finally, we show experimentally the successful amplification of DNA-molecules with microwave fields.
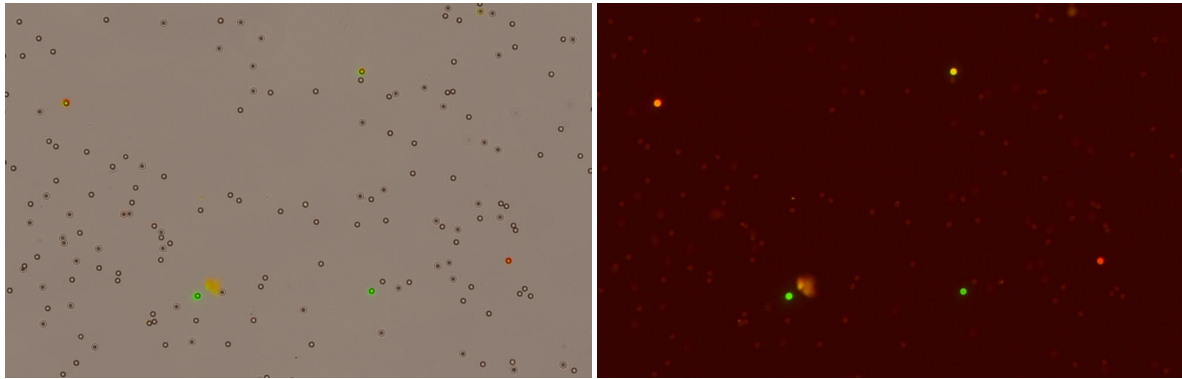

Figure 5: Images of arrayed beads after bead-emulsion amplification, using the microwave probe. The right panel represents an overlay of two images taken in two different fluorescent channels of an epi-fluorescence microscope, in the left panel additionally a brightfield image of the beads is overlaid. Only a fraction of the beads carried an initial single molecule and resulted in a fluorescent bead covered with amplicons.

For the present investigation bead-emulsion amplification (BEA) as described in [3] was chosen to proof experimentally the feasibility of using microwave heating components to amplify single molecule DNA. In BEA single DNA-molecules are attached to microscopic paramagnetic beads compartmentalized in an emulsion. At the beginning of the amplification experiment the incident microwave power P_{1+} was continuously raised until the thermocouple near the liquid surface indicated a temperature of ~50°C. In the present case an incident power P_{1+} of 32dBm at 2.1GHz was needed, and equilibrium was reached within a few minutes. After approximately one hour treatment with the microwave probe, the amplification efficiency was measured in terms of fluorescent signal intensity. Figure 5 illustrates the efficient amplification of single molecules evaluated by fluorescent probes annealing to the amplified material. Beads fluorescing brightly represent successful single PCR amplification. The resulting intensity (representing to the number of DNA molecules amplified on a bead) was compared between the microwave probe and a standard thermocycler. Beads amplified with a standard thermocycler had a ~7.5 increase in intensity compared to ~6.5 increase obtained using the microwave setup. For both amplification processes, similar numbers of beads with a product were recovered.

CONCLUSION

In conclusion we have shown that convective PCR for mobile lab-on-a-chip devices can be realized just by using standard power RF-components, originally developed for wireless communication applications. A mobile RF-oscillator plus a downstream amplifier and an open-ended coaxial cable as a microwave probe are sufficient to realize convective PCR in a standard microtube, without the need of additional cooling or heating elements. Thus the proposed setup is suitable for lab-on-a-chip developments also in other technologies.

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CONTACT

*W. Hilber, tel: +43-732-2468-6259; wolfgang.hilber@jku.at