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ARTICLE

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Rapid PCR amplification of DNA utilizing Coriolis effects

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Abstract A novel polymerase chain reaction (PCR) method is presented that utilizes Coriolis and centrifugal effects, produced by rotation of the sample disc, in order to increase internal circulatory rates, and with them temperature homogenization and mixing speeds. A proof of concept has been presented by testing a rapid 45-cycle PCR DNA amplification protocol. During the repeated heating and cooling that constitutes a PCR process, the 100 μL samples were rotated at a speed equivalent to an effective acceleration of gravity of 7,000 g. A cycle time of 20.5 s gave a total process time of 15 min to complete the 45 cycles. A theoretical and numerical analysis of the resulting flow, which describes the increased mixing and temperature homogenization, is presented. The device gives excellent reaction speed efficiency, which is beneficial for rapid PCR.

Introduction

As the most widely used DNA amplification method in the life sciences, the polymerase chain reaction (PCR) has been the object of much effort concerning process optimization. Several attempts to speed up the process have been made. The most obvious and commonly applied strategy to obtain rapid temperature ramping has been to reduce reaction volumes combined with designing reaction vessels like capillaries (Wittwer et al. 1989), cuvettes (Columbus et al. 1990; Petersen et al. 1999), silicon-based sleeves (Northrup et al. 1996), vortex-tubes (Ebmeier et al. 2004), pipette tips (parallabs), ultra-thin-walled microplates (Hermann et al. 2004) and various kinds of microfluidic devices. Unfortunately, such approaches to reduce the reaction volume, directly compromise the potential for developing the highly sensitive PCR assays often needed for detection and quantification of for example infections agents. The reason for this is that inhibitors originating from the sample itself, e.g. blood, urine or food, often have a detrimental effect on the PCR efficiency. However, a high volume PCR (such as 100 μL), dilutes the inhibitors more efficiently which increases the possibility to detect the infectious agent.

For PCR-reactions in conventional volumes (20–50 μL) and industry standard PCR tubes, the limits for speeding up ramping rates are currently set by the time it takes to homogenize the temperature in the reaction mixture. Here we report on a new thermal cycler in which thermal homogenization is rapidly obtained by the introduction of a complex three-dimensional internal motion of the reaction mixture through the rotation of PCR tubes mounted in a disk-formed rotor, see Fig. 1 and Malmquist et al. (2004). A 45-cycle PCR DNA amplification protocol that was implemented has proven the method effective. Fluorescence levels representing accumulating numbers of PCR-fragments from 48 real-time PCR-reactions are monitored simultaneously during the rotation. Extensive numerical simulations have been performed to understand the enhanced mixing capabilities of the novel design.

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Fluid mechanics

In order to understand why the proposed method produces an excellent PCR product, and also to enable further improvements, a look at the physics behind the motion of the PCR mixture in the tube was necessary. The liquids used for PCR analysis typically contain relatively low concentrations of template DNA. The

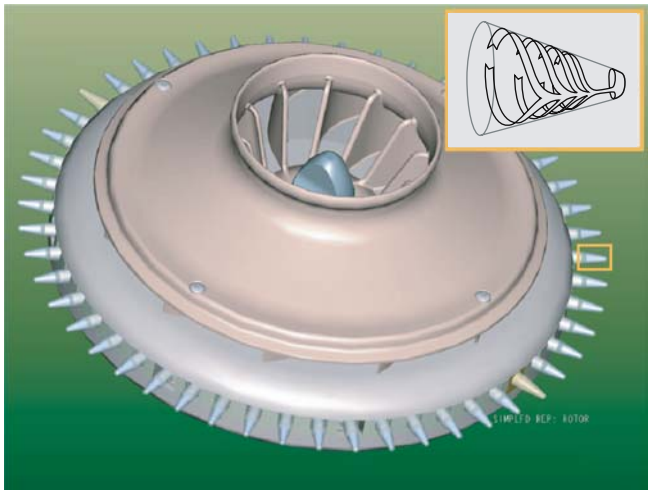

Fig. 1 The tube is mounted horizontally on the 48-sample disc and rotated parallel to the symmetry axis of the disc. Schematic details of the boundary layer driven internal flow, that is induced by rotational effects and carries fluid along the surface of the tube and forces it towards the apex, are shown in the inset

material properties can therefore be reasonably well described by the Newtonian fluid model, where viscosity is taken to be a constant. Since the pieces of DNA being duplicated are small, the effects on the viscosity (compared with water) are negligible or small.

The basic equations of motion for an incompressible Newtonian fluid are the Navier-Stokes equations. In order to capture the effects of buoyancy, i.e. the forces acting on the fluid due to a variation in the density, the density is described as a function of the temperature in the centrifugal force term. The temperature field is coupled to the velocity field through the energy equation, which describes the evolution of the temperature distribution.

A rudimentary analysis of the governing equations yields three important features of the flow. First, due to the size of the tube and the estimated flow speeds, the flow will be *laminar*, i.e. no turbulent flow will evolve. Second, a scaling analysis yields that the magnitude of the velocity is linearly dependent on the rate of rotation, see Skote et al. (2005). Third, the ratio between inertial and rotational forces tends to become independent of the rotation rate. Thus, even though the velocity increases with rotation rate, no radical changes in the character of the flow field will evolve by increasing the rate of rotation, at least up to the limit where the flow becomes turbulent.

The relations above can be deduced from the non-dimensionalized equations of motion, where the so-called Grashof, Rayleigh, Ekman and Rossby numbers appear. The magnitude of these non-dimensional numbers determines the character of the flow. For instance, the third feature mentioned above is related to the fact that in this case the Rossby number tends to a finite constant as the rotation rate is increased. The Rossby number, ε is defined as $U/\Omega d$, where U is a characteristic speed of the flow, Ω is the rate of rotation and d is a characteristic length tied to the flow.

Materials and methods

Rapid PCR

A 45-cycle PCR-protocol has been used to test the validity of the proposed prototype. Samples were set up in 100 μL volumes containing (final concentrations): HER-2 gene specific primers 300 nM each, SYBR Green I (Sigma) diluted 1:70,000, 2.5 U Taq polymerase (Sigma), 1× PCR buffer (Sigma), 3 mM $MgCl_2$ (Roche), 0.2 mM of each dNTP (Amersham Bioscience) and template DNA (cDNA prepared from human breast tumor total RNA). The efficacy of the process was studied at different concentrations of template DNA, starting from 2.5×10^4 copies up to 4.0×10^5.

The conventional 0.2 mL polypropylene PCR tubes (Microplast AB, Skara, Sweden) are attached horizontally in the PCR device and subjected to an elevated g-force (7,000 g) through the rotation of the sample disc. Heating of the PCR tubes is performed with a custom built infrared heating apparatus. Effective cooling is attained using the rotational motion of the sample disc, which acts as a fan, sucking air through the apparatus. The heating schedule consisted of 45 consecutive cycles of denaturing and a combined annealing/extension step, 5 s of denaturation at 98°C followed by 8 s at 70°C for the first four cycles. Followed by 1 s of denaturation at 95.5°C and 5 s at 70°C for the remaining 41 cycles.

This cycling process produces an amplicon 120 bp in size. The annealing temperature is fully flexible in terms of temperature 'adjustability' and may be set to 0.1 C tolerance.

Simulation-code

The simulations are performed with the commercial code CFX run in double precision on a Linux system at the Department of Mechanics, Royal Institute of Technology (KTH). All results have been shown to be grid-independent. The coupled equations governing the flow and temperature distributions are discretized and solved numerically using a finite-volume method on a grid that typically consists of 150,000 volume elements.

Results and discussion

Rapid PCR

A 45-cycle PCR DNA amplification protocol, targeting a short region (120 bp) of the HER-2 gene, was implemented to test the new method. Five dilutions of

the target DNA (1:5, 1:10, 1:20, 1:40 and 1:80) and a negative cDNA test target were amplified. In Fig. 2, the amplification of the different target products is monitored by the fluorescence of the double-stranded DNA specific dye SYBR Green I. Two curves for each dilution concentration are shown to illustrate the reproducibility of the process. It is easily noticed that amplification is dependent on concentration, producing an earlier amplification start with increased concentration of target DNA. It may also be observed that a lag of one cycle is present with the decrease of initial concentration by a factor two. The positive NTC is due to primer dimer artefacts.

The corresponding agarose gel (2%) for the five concentrations and control reactions is presented in Fig. 3. It is clear from this data that an effective PCR has been performed resulting in highly amplified product for all concentrations.

Effects of rotation

Although the beneficial effect of rotation of the PCR tube is supported by the rapid PCR described above, understanding the necessary inner mechanisms demands a deeper physical probing. For simplicity, the flow will be described in the rotating frame of reference. There are two additional forces acting on the fluid in the rotating system: the centrifugal force and the Coriolis force. The former is responsible for setting up a hydrostatic-like pressure gradient through the fluid. In the case of a non-rotating fluid, gravity is the sole body force, which sets up the pressure gradient. In this fast rotating application however, the centrifugal force is much larger than the gravity, which therefore can be neglected ($\Omega^2 R >> g$).

The Coriolis force is in this case responsible for turning the flow in the azimuthal direction. An effect of this force in nature is e.g. the curving of the trade winds due to the rotation of the earth.

In this strongly rotating system the flow field exhibits features, which could never arise in a non-rotating system. However, the flow is not completely governed by rotation; the non-linear inertial part of the Navier-Stokes equations is important regardless of how large the rotation rate is. This is related to the finite non-zero Rossby number of this particular flow.

Boundary conditions

The cooling rate depends on the boundary conditions (BC) for the heat transfer from the surrounding air to the fluid inside the tube. In the simulations presented here, we do not include the plastic tube, i.e. the wall is considered infinitesimally thin implying direct contact between the fluid inside and outside the cone. If a fixed temperature is set as a BC, the heat transfer is infinite, and the temperature of the fluid closest to the wall will immediately attain the value set by the BC.

The heat transfer through the wall is possible to model through a BC in which the heat transfer coefficient (h) is set. The heat transfer, $q'' = h\Delta T$, will then depend on h and the temperature difference (ΔT) between the surrounding air and the fluid closest to the wall.

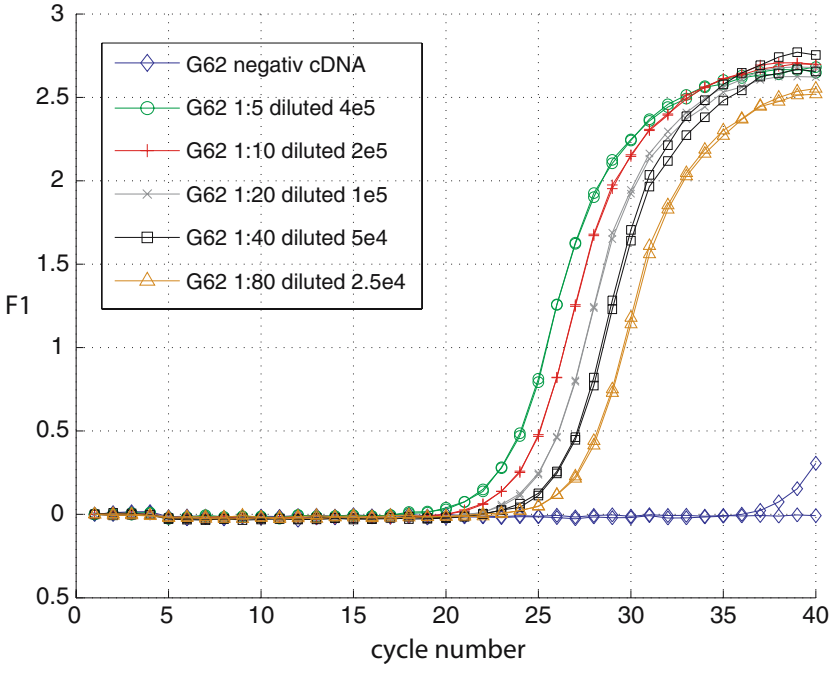

Fig. 2 Fluorescent monitoring of rapid cycle PCR DNA amplification after compensating for the baseline signal. The target was a 120-bp region of the HER-2 gene. Samples were cDNA prepared from human breast tumour total RNA. Initial product concentrations from 2.5×10^4 copies up to 4.0×10^5 of G6PDH 2 and cDNA are presented. Two negative samples of cDNA were also included

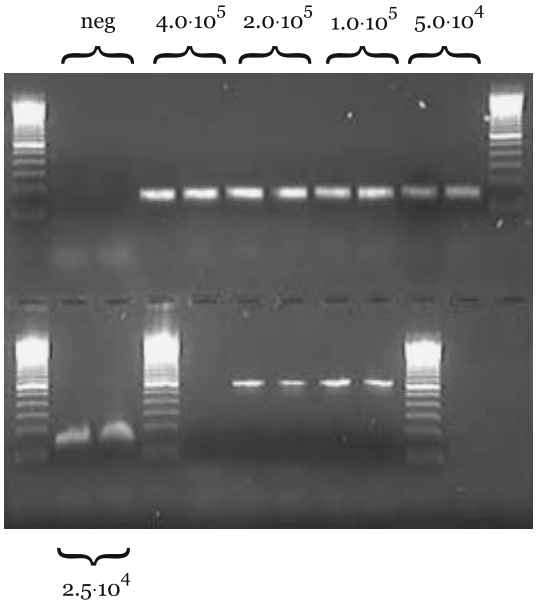

Fig. 3 An agarose gel stained with ethidium bromide and illuminated with UV light which causes the intercalated stain to fluoresce. The five concentrations, as well as the negative samples, from the PCR are labelled in the figure, while 100-bp ladders and control reactions are unlabelled

Flow field

In the rapidly rotating PCR tube geometry, we have a flow that we will refer to as *superconvection* and that will be dominated by mass transport in thin boundary layers near the walls of the tube. Furthermore, the main direction of the flow in these boundary layers is in the azimuthal direction. This is a strikingly non-intuitive result.

If we consider the non-rotating case with the acceleration of gravity directed towards the apex of the tube, and an ambient temperature lower than the fluid, the cooled fluid elements would be driven downwards along the whole inside of the tube towards the apex.

This is in sharp contrast to the rapidly rotating case, where the main flow in the boundary layer is driven along the wall in the azimuthal direction towards the horizontal mid-plane, where a concentrated rapid stream is set up that will transport the cooled fluid towards the apex of the tube (the "effective" bottom of the tube).

Heating

The heat is distributed through infrared radiation and is modelled in the simulation by a source in the energy equation confined to a volume close to the apex.

The flow that evolves is complicated and three-dimensional, but the main feature is the flow upward from the apex, which is concentrated to a rapid flowing stream located close to the wall. Instabilities of the flow create rapid fluctuations, and subtle breaks in the symmetry of the flow, which enhance the mixing of the fluid and hence rapidly gives a uniform temperature, a necessity for reliable and quick PCR. Without the rotation, the heating would be less effective since it is the strong force field created by the rotation that makes the buoyancy effects so powerful.

Cooling

The flow is triggered by the difference in the temperature. When the fluid is cooled close to the wall, it is initially driven towards the apex because of the equivalent acceleration of gravity in that direction due to the rotation, see Fig. 4. This transient flow field is sustained only for a short period of time. The results presented are for simulations performed for $\Omega = 6{,}000$ rpm, but calculations at higher rotation rates show only small qualitative differences in behaviour.

After about half a second, a semi-stationary flow field is established where the downward flow is concentrated to one side of the tube, see Fig. 5. The Coriolis force is responsible for this behaviour. The flow towards the apex along the wall in Fig. 4 is turned to a flow in the azimuthal direction due to the Coriolis force, see Fig. 6. The two boundary layers are flowing in the same direction and meet in a region where the flow turns towards the apex of the tube (see also Fig. 1). After this flow has been set up, the flow pattern remains quasi-stationary, with a magnitude of the velocity that diminishes as the temperature difference decreases.

We can then see that the Coriolis force creates a flow which is completely different from that in a non-rotating case, where the downward flow would be equally large around the wall with the upward flow concentrated in the middle of the tube in an axisymmetric pattern. The azimuthal flow would be zero in this case.

In the quasi-stationary situation, the Coriolis force is approximately balanced by buoyancy. This gives a relation for the azimuthal velocity (v) (near the wall) that can be written as

$$v \sim \Omega R \beta \Delta T \tag{1}$$

from which we can see the linear dependence on rotation rate (Ω). In (1), R is the distance from the axis of rotation (approximately 0.10 m in this case) and β is the volumetric expansion coefficient of the fluid (water at 20°C). We can also observe the dependence on the driving temperature difference (ΔT), i.e. a decreasing velocity with decreasing ΔT. An order of magnitude analysis for $\Omega = 10^4$ rpm (approximately 10^4 g) yields an azimuthal flow speed of circa 1.5 m/s in the boundary layer. Hence, very high velocities will occur in the boundary layer driving a high rate of mass transport thereby enabling a very rapid homogenization of the fluid temperature in the PCR tube.

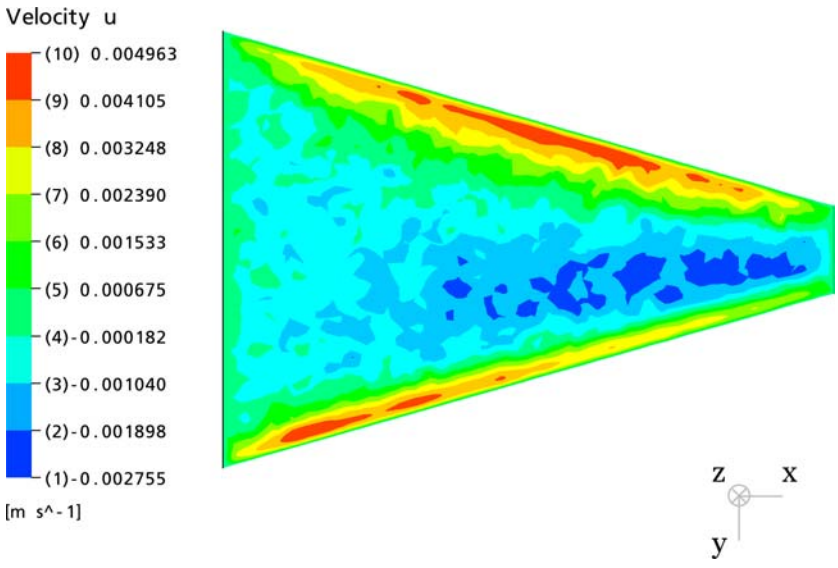

Fig. 4 The flow field after 0.1 s of cooling. The view is from below and the rotation counter-clockwise. *Red* colour denotes flow towards the apex. The rotation rate is scaled down to $\Omega = 100\ s^{-1}$ which is equivalent in this case to 100 g

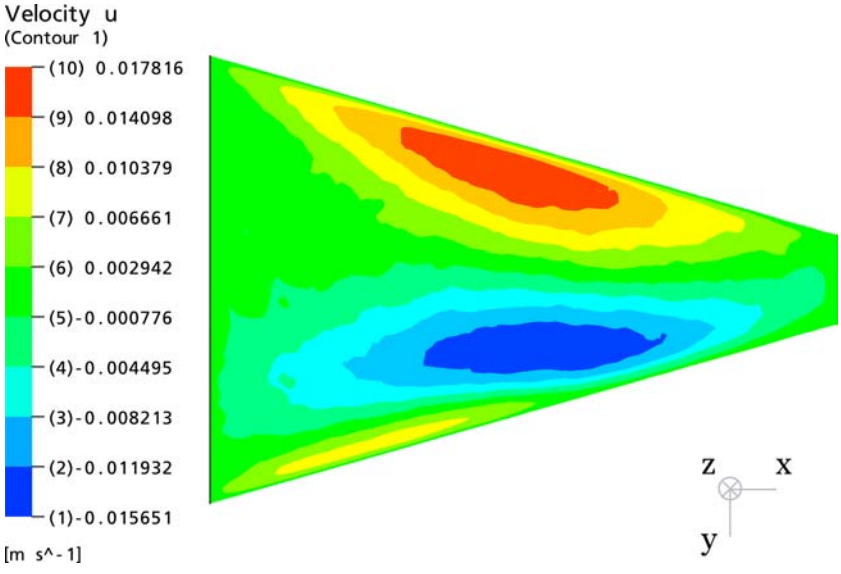

Fig. 5 The flow field after 0.5 s of cooling. The downward flow is concentrated to the lee side. *Red* colour denotes flow towards the apex. The rotation rate is scaled down to $\Omega = 100\ s^{-1}$ which is equivalent in this case to 100 g

Isothermal conditions

During chemical processes under isothermal conditions, the mass transport in the liquid is of great importance. If the isothermal conditions are set by asymmetric cooling from the wall and heating through radiation simultaneously while rotating the system, the relation mentioned above shows that a substantial mass transport would occur in the liquid. Thus, even under isothermal conditions, but with a $\Delta T \sim 10°C$ there is much to be gained by letting the system rotate.

Comparison with other techniques

With a more traditional system of non-rotating tubes immersed in a heating/cooling block, the velocities of the liquid will be several orders of magnitude lower than in the rotating case. Since the dominant mechanism for obtaining a uniform temperature is convection, the small velocities result in a slow temperature homogenization and poor mixing.

In the case of capillary tubes, the dominant mechanism for heat transfer is diffusion. The pipes are narrow enough for the diffusion to spread the temperature over the cross-section before the convection is fully developed. In order to maximize the heat transfer, the area to volume ratio should be as large as possible. Since this relation varies with the diameter (D) as $\sim 1/D$, the diameter should be as small as possible. Another positive effect from a small diameter is that the diffusion time-scale will decrease, i.e. the heat diffuses faster over the cross-section. However, the volume of interest must be retained and the length must be kept under some

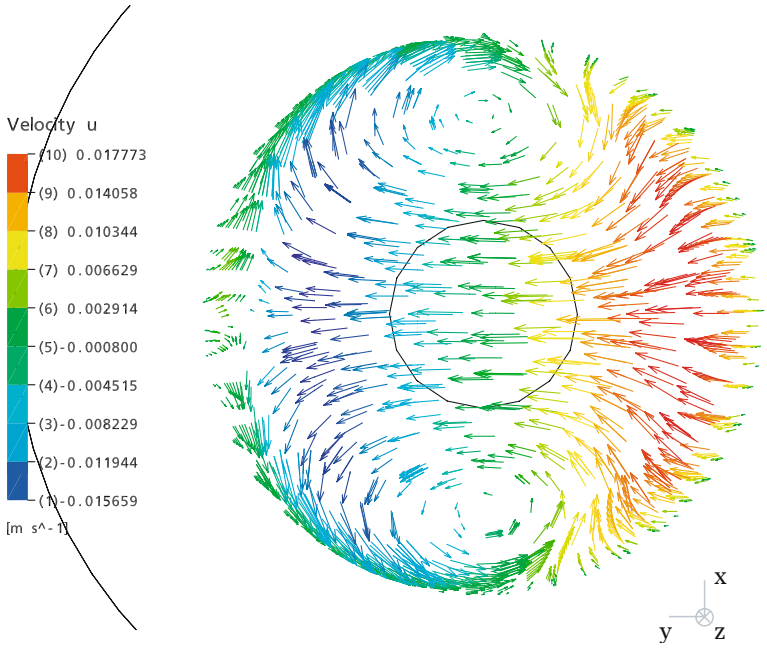

Fig. 6 The same flow field as in Fig. 5, shown in a cross-plane taken through the middle, looking towards the apex. *Arrows* represent velocity in the plane, while *colours* represent the velocity in the third direction (*red* colour denotes flow towards the apex)

limit. The ratio of diameter/height can be calculated for the balance between convection and diffusion described above to be valid.

The disadvantage inherent to capillary methods is that little or no mixing occurs during the process and that only relatively small volumes can be considered. The concept with tall and thin pipes also negatively affects the handling of the sometimes vast amount of samples. Large volumes will also be troublesome since the taller the pipes get, the more easily they will break, and handling can be cumbersome.

If one reduces the length and increases the radius, the convection becomes more important than diffusion. By increasing the effective gravity obtained through rotation, the convective time-scale is reduced (velocity is increased). However, stratification is an inherent problem in this case.

Summary

A novel method of rapid PCR DNA amplification utilizing Coriolis and centrifugal effects tied to system rotation of the PCR tube sample disc has been presented. A proof of concept trial PCR was performed where DNA at different concentrations was amplified in a standard 45-cycle process, where each cycle was only 20.5 s long. The rotation-based PCR method makes it possible to obtain a very fast and at the same time very efficient amplification process, even for a 100 μL reaction.

A theoretical and numerical analysis of the internal flow of fluid in the PCR tube has unveiled an intricate flow dependent on rotational effects. A secondary flow normal to the axis of symmetry of the tube is induced by the Coriolis effects. This flow, along with the flow towards the apex, increases the rate of temperature homogenization, which in turn makes a shorter PCR cycle realizable. It is also believed that the increased internal motion causes a more effective mixing of ingredients of the PCR mixture resulting in a more efficient product amplification.

Temperature measurements inside the tube during PCR-cycling further confirm the computational results regarding the temperature homogenization. Further details of the flow analysis and computational results, including temperature distributions, are to be reported in Skote et al. (2005).

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