A Project Report

On

Microfluidic on-chip Polymerase Chain Reaction (µPCR)

BY

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2013B1AB844H

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SUBMITTED IN PARTIAL FULLFILLMENT OF THE REQUIREMENTS OF

BIO F376: DESIGN PROJECT

BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE

PILANI (RAJASTHAN)

HYDERABAD CAMPUS

(December 2016)

ACKNOWLEDGMENTS

I would like to thank my alma mater, BITS-Pilani, Hyderabad Campus, for providing me with an environment conducive to learning and the resources required for this study.

I would like to express my heartfelt gratitude to Dr. K.N. Mohan for guiding me and providing me with useful insights and expertise.

I would like to thank Dr. Sanket Goel and his Ph.D. students, Ms. Smrithi Ajit and Mr. Puneeth, for their invaluable suggestions and inputs.

Last, but not least, I am grateful to my parents and friends for their constant support.

Birla Institute of Technology and Science-Pilani,

Hyderabad Campus

Certificate

This is to certify that the project report entitled

"Microfluidic on-chip Polymerase Chain Reaction (µPCR)"

submitted by Mr. Abhishek Dave (ID No. **2013B1AB844H**) in partial fulfillment of the requirements of the course BIO F376, Design Oriented Project, embodies the original work done by him under my supervision and guidance.

Date: December 2016

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ABSTRACT

The Polymerase Chain Reaction, or PCR, was first reported by Dr. Kary B. Mullis in 1983. It is today the standard technique for amplifying DNA and has far-ranging applications, from forensics to diagnostics. However, the growing demand for cheaper, faster, and smaller PCR devices has catalyzed the interest in manufacturing microfluidic devices capable of conducting PCR.

The spurt in the on-chip biochemistry industry has seen the creation of a variety of complex technologies, from lab-on-a-chip to cell- and even organ-on-a-chip systems. The PCR-on-a-chip systems often include both pre-processing and post-processing of the DNA samples, i.e. cell lysis and DNA purification, as well as further electrophoresis (after amplification) to validate the results of PCR.

While the aim of microfluidic PCR is to enhance and enable cheap and easy PCR, its manufacture poses several challenges. The risk of contamination must be controlled and minimized, the samples must be heated to an appropriate extent for an ideal duration, and the amplification must be measurable. This report aims to address these subjects, discussing the various technologies that have been developed to tackle the issue of heating, contamination, etc. Later work during the project has been in simulating actual PCR-on-a-chip models in COMSOL Multiphysics 5.2.

A variety of geometries were explored before selecting the triangular (and later toroidal) geometries that are presented herein.

Ultimately, microfluidic PCR presents a unique opportunity to disrupt the status-quo in on-site diagnostics – a very valuable one, in light of recent epidemics such as Zika, the avian flu, etc.

Here we have explored natural convective flow driven PCR in two potential geometries, one of which has been realized into a prototype in the lab.

KEY WORDS: PCR, microfluidic, on-chip PCR, lab-on-a-chip, natural convection flow.

OBJECTIVE

The objective of this report is to address the foundational principles of microfluidic polymerase chain reaction – how these devices are built, the materials that can be used to manufacture them, and most importantly, the techniques being utilized to generate the temperatures required for PCR to be conducted.

A variety of materials, from glass and silicon, to polymethylmethacrylate (PMMA) and polydimethylsiloxane (PDMS) are used to make the chips, with varying properties to suit the needs of individual studies and applications.

Heating is an equally diverse area of interest, with several methodologies being adopted to heat microfluidics chips, from lasers and microwaves to Peltier junctions.

This report will discuss the benefits and drawbacks of these options within each parameter that has been identified (material, heating technique, etc.)

The final part of this report, and project, will focus on the practical component therein – i.e. COMSOL Multiphysics simulations of natural convection PCR in multiple geometries.

CONTENTS

Chapter 4 : Simulation Work

After a thorough literature survey, it was determined that a closed loop geometry, with natural convection-driven flow (i.e. fluid flow established by virtue of temperature gradient between different regions), would be the most suitable for our prototype. The paper we referred to was “A Pocket-Sized Convective PCR Thermocycler”, Agrawal et al, Angew. Chem. Int. Ed. 2007, 46, 4316 –4319.

The reason for choosing natural convection driven flow over other flow regimes, such as peristaltic pump driven flow, was to develop

- A cost-effective, self-sustained model that takes advantage of channel geometry to achieve continuous unidirectional flow
- Experience minimal compounding of error (more independent, complex systems means that errors in each get summed up)

Accordingly, the first model that was simulated was a closed-loop triangular channel with equilateral geometry.

The premise behind this geometry was simple –

- One side of the equilateral triangle would be heated (by positioning a Peltier plate apparatus along the perimeter) to ~95°C, corresponding to the first step of PCR, i.e. denaturation.
- The immediately adjacent face would have another Peltier apparatus providing ~55°C temperature to the channel (correspoding to the annealing phase of PCR).
- Lastly, the extension phase of PCR (occurring at ~ 72°C) would be allowed to occur passively - by the establishment of a natural thermal gradient in the last arm of the triangular channel, meaning that no additional Peltier aparatus would be required there.
- Essentially, the temperature gradients from the other two arms neutralize each other in the third arm, leading to a zone of intermediate temperature, suitable for the extension phase to take place.

Mathematical framework:

In order to develop an accurate simulation, we had to first explore the mathematical assumptions and boundary conditions etc. In this regard, the study of buoyancy driven natural convective flow was conducted.

The buoyant force on the fluid is :

$$F_B = \int_0^L (\rho_s - \rho) A \cdot g \cdot \cos\theta \cdot dl \qquad (1)$$

where rho is the density; rho_s is the reference fluid density at the surrounding temperature; A is the cross-sectional area of the channel; L is the length of the loop channel; g is the gravitational acceleration; l is the directional coordinate and theta is the angle between the direction of the flow and gravity.

Following this, we need to make the Boussinesq approximation for incompressible fluid flow (water)

$$F_B = \int_0^L \rho_s \beta (T - T_s) A \cdot g \cdot \cos\theta \cdot dl \qquad (2)$$

where T is the temperature of the fluid; Ts is the surrounding temperature; and beta is the thermal expansion coefficient.

The scaling of viscous shear force in the channel flow can be expressed as:

$$F_S \sim \frac{\mu VPL}{R_h} \qquad (3)$$

where mu is the dynamic viscosity of fluid; V is the flow velocity; P is the perimeter of the channel; L is the length of the loop channel; and Rh is the hydraulic radius (Rh = A/P) which is a characteristic length scale of the channel.

Following this, we need to strike a balance between the driving buoyant force and the counteractive viscous shear force. From this analysis, we obtain the speed and circulation time for buoyant flow in the loop.

$$V \sim \frac{(g\cos\theta)\,\beta\ \Delta T\ R_h^2}{\nu} = \frac{Ra\cdot\kappa}{R_h} \qquad (4)$$

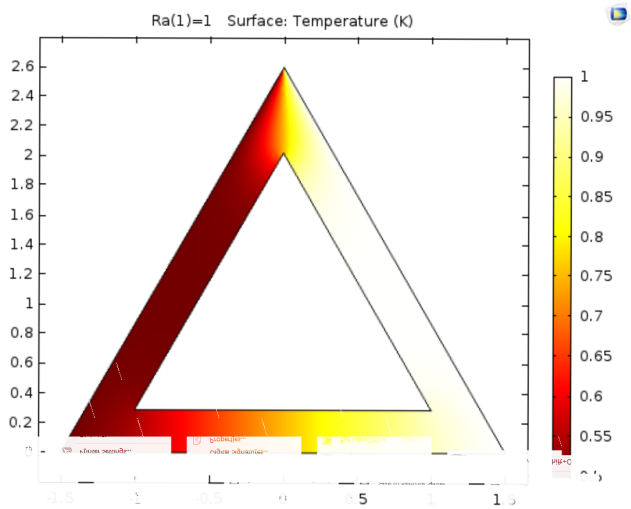
$$t \sim \frac{\nu L}{(g\cos\theta)\,\beta\,\Delta T\,A} = \frac{R_h\cdot L}{Ra\cdot\kappa} \qquad (5)$$

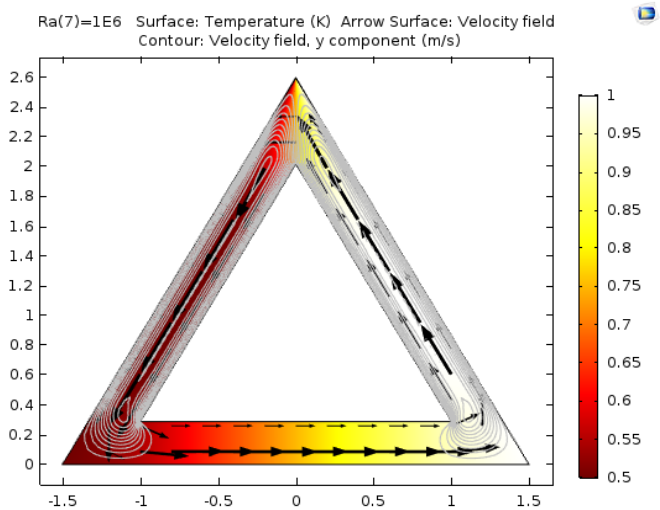
Finally, Ra, the dimensionless Rayleigh number, which characterizes the relative magnitude of buoyancy forces against the diffusive viscous forces, is defined as:

$$Ra = \frac{(g\cos\theta)\,\beta\ \Delta T\ R_h^3}{\nu\kappa} \qquad (6)$$

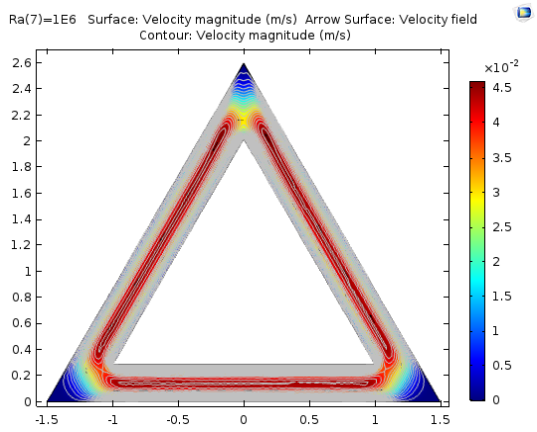
Approximations of the above framework are implemented in COMSOL

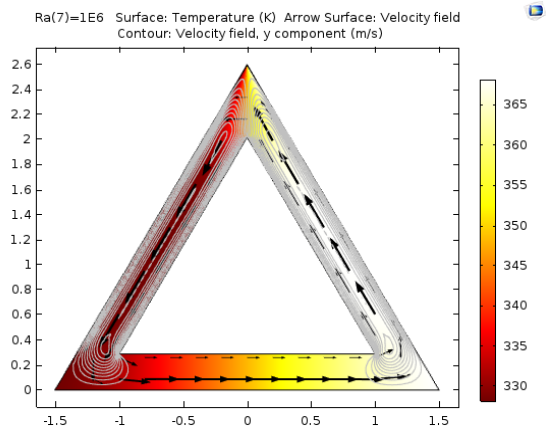
For the first model, a nondimensional model was assumed, as shown below. Temperature was provided only on the outer perimeter of the channels, following the approach of Agrawal et al.



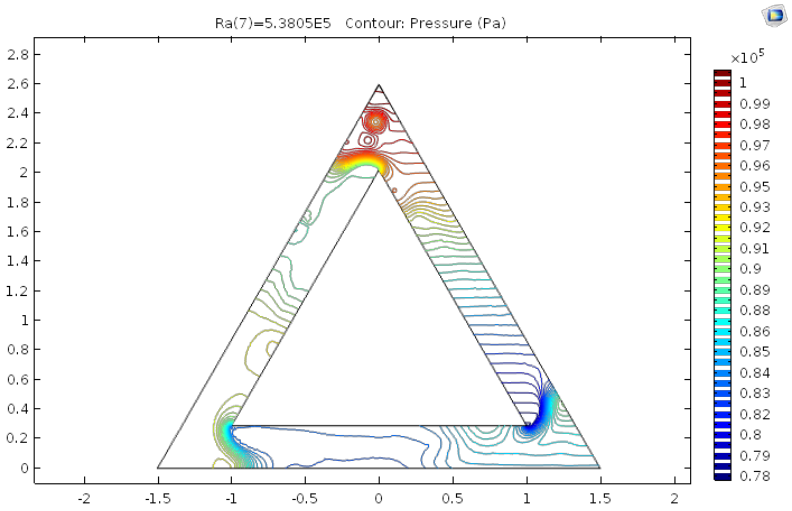


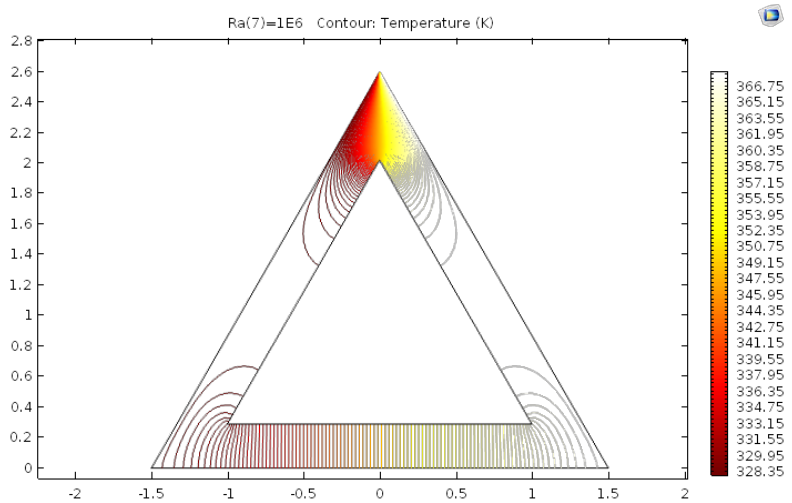
In the second iteration of modelling, dimensionality was restored by taking actual values of all parameters. Certain parameters like Cp were estimated by finding their values at the mean temperature in the triangular channel (~58°C).

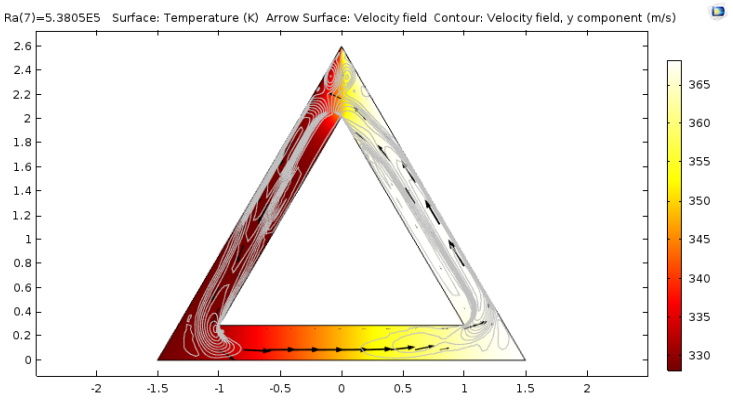


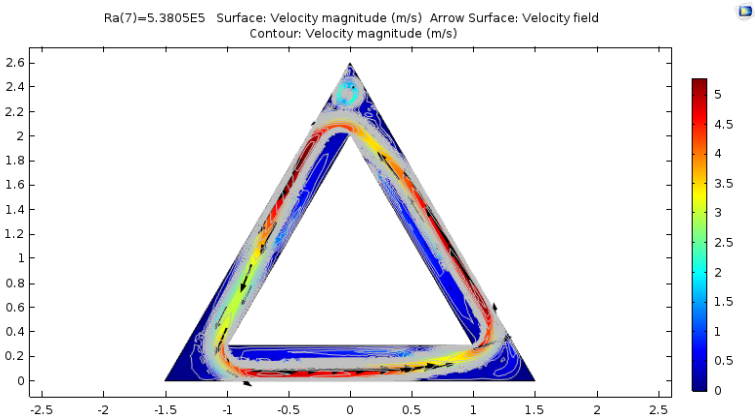


In the final iteration of sharp triangular geometry, we were able to simulate the following results by refining and adjusting some of the software features.



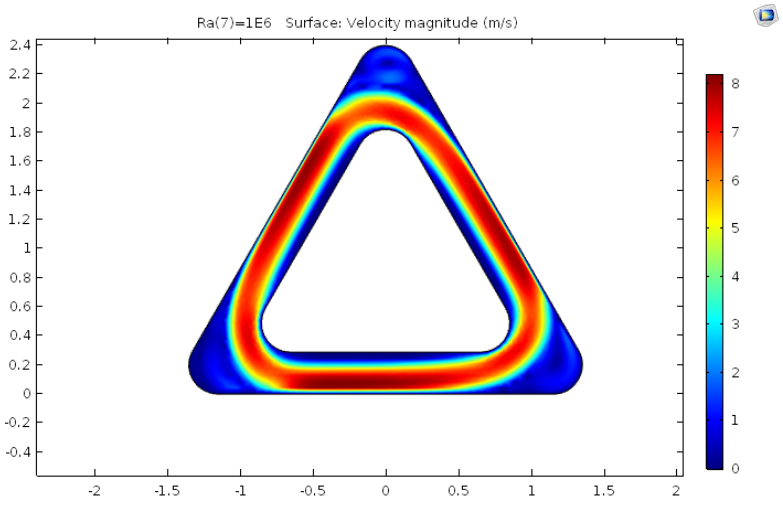


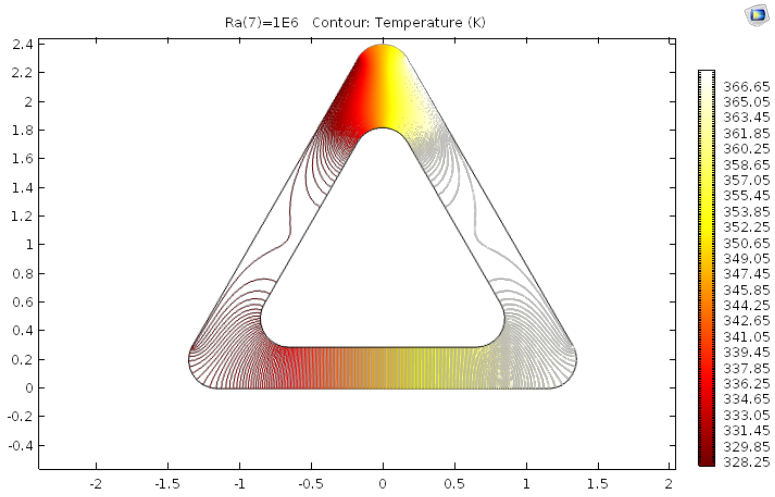


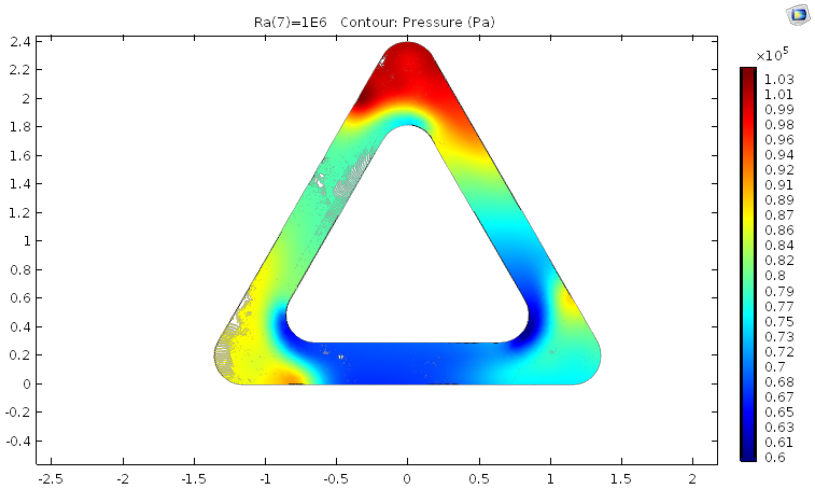


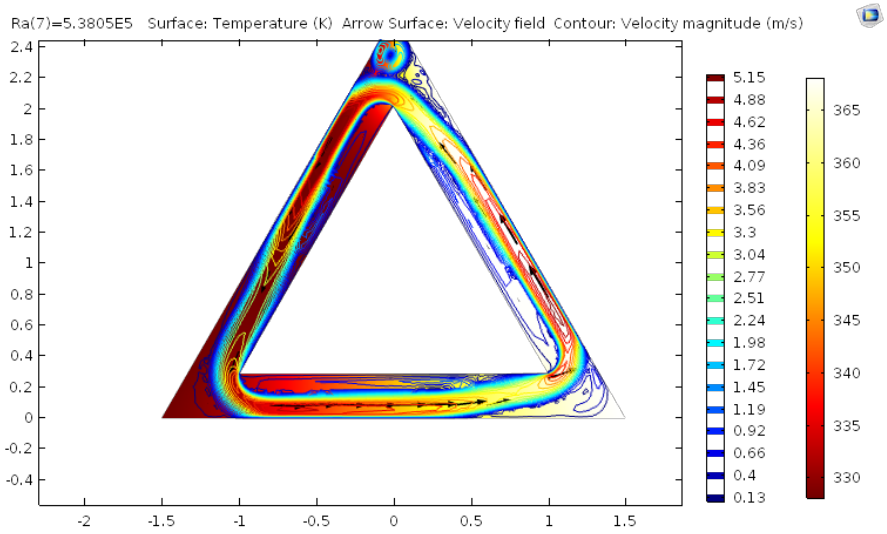
However, since the practical setup requires the use of silicone tubing to create the channels, it is not feasible to have a sharp corner geometry. Also, the presence of sharp corners increses the possibility for local vorticities of flow to occur. These are a waste from our perspective, and hence need to be eliminated.

Hence, the corners of this geometry were filleted with a radius of 0.2 to yield the final triangular channel model, as shown below.



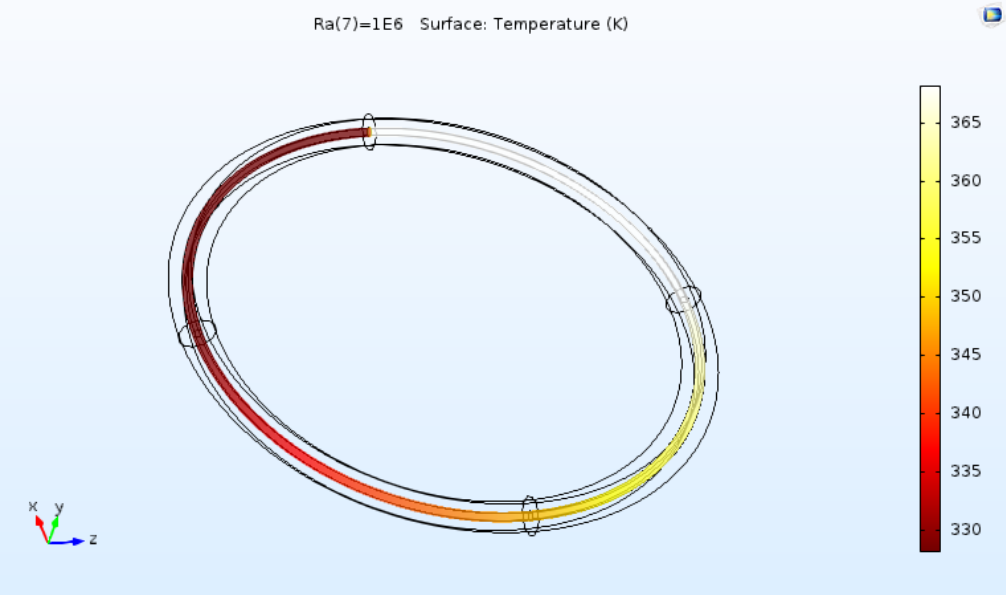


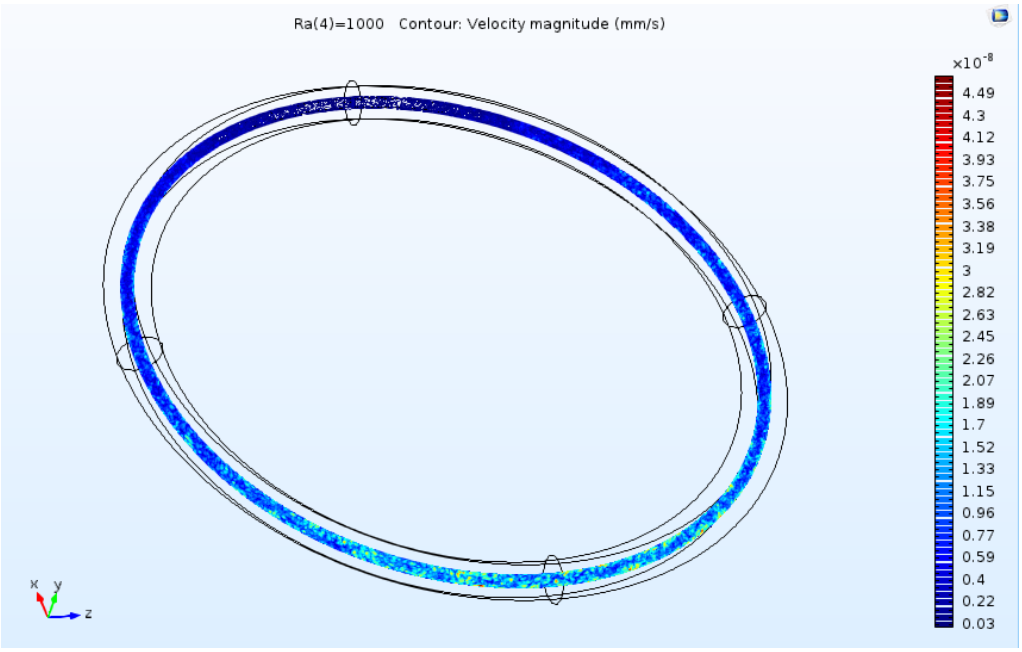




However, owing to constraints in the experimental setup, the triangular model had to be scrapped. Instead, a toroidal model was proposed, with a similar heating scheme and flow regime. The results for the toroidal

simulation are depicted below.





The above has also been implemented in the laboratory, and is being developed as a proof-of-concept for this model of natural convection-driven flow for PCR in a microfluidic device.

Conclusion

There is a growing global awareness of the need to develop fast, efficient, and inexpensive technologies for disease diagnosis, especially in resource-starved settings. Accordingly, a large amount of funding is being allocated to projects that pave the way for creation of such devices.

The microfluidic/MEMS platforms for diagnosis represent a significant contribution to these efforts, with hundreds of innovative methods which build upon advances in chip-fabrication and apply it to real-world problems of diagnosis.

This study has discussed the fundamental principles of PCR in the context of designing a microfluidic chip-based PCR system – including the dual challenge of temperature control and contamination of sample.

This study has also demonstrated several simulations of natural convection-driven microfluidic PCR in two geometries, namely triangular and toroidal. The toroidal geometry has also been implemented in the lab using silicone tubing, Peltier elements, and Arduino microcontroller.

Looking to the future, the development of innovative and indigenous devices that harness the tools of microfluidics is a necessary step to counter the threat of epidemics and pandemics like Ebola, Zika etc.

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