Modelling of Serpentine Continuous Flow Polymerase Chain Reaction Microfluidics

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Abstract

The continuous flow Polymerase Chain Reaction (PCR) microfluidics DNA amplification device is a recent discovery aimed at eliminating the cyclic hold experienced while using the alternative stationary device. The Application of Computational Fluid Dynamics is increasingly growing and can help achieve optimal designs before actual fabrication. This paper presents a CFD modelling of a continuous flow serpentine PCR device with narrow and wider channels. There are two temperature regions at 95^0C and 60^0C for denaturation and annealing respectively. Extension is achieved along the middle of the channel at 72^0C owing to temperature gradient. The model require a pressure of 42.6KPa for a 30 cycle amplification.

Keywords: *PCR, CF-PCR, DNA Amplification.*

1.Introduction

The development of Polymerase Chain Reaction (PCR) and its distinct quality to exponentially amplify a deoxyribonucleic acid (DNA) sequence sample [1] makes it a source of concern for engineers to devise the most efficient method of its application. PCR is performed when the sample mixture passes through three temperature regions to carry out denaturation, annealing and extension of the specific target sequence. A single cycle has three stages: In the first stage, the sample is heated to a temperature of ~95^{o}C to let a double-stranded DNA molecule to be denatured into a two single-stranded DNA molecules; In the second stage, the sample is cooled to a temperature of ~60°C for annealing of the primers to the single stranded DNA template; In the final stage, the temperature is raised again to ~75°C for extension of the targeted DNA in the presence of a polymerization enzyme and deoxynucleotide triphosphates [2]. This processes needs to be done as quickly as possible due to the importance of its application in monitoring [3] and diagnosis of serious infectious diseases [4]. Achieving a high speed PCR in a conventional PCR device has been difficult due to the fact that the device is made of metal block. The rate of heat transfer to and from the sample is determined by the rate at which the device is able to absorb and dissipate heat. The thermal mass of the PCR device therefore limits the speed at which PCR is performed. In addition to the large thermal mass, the conventional PCR has high consumption of expensive reagents and a potential for sample contamination. A complete PCR amplification in a conventional PCR device based on the metal block with 20–40 cycles will usually take 1–3hrs because thermal ramp is at the range 1-2^{o}C/s [5, 6]. Most of that time is used for the heating and cooling of the PCR mixture due to the need to bring the large metal block to the cycling equilibrium temperature and to transfer heat to the PCR mixture. It therefore means that the PCR cycling speed can be increased by mainly developing a desirable PCR device that is capable of acquiring fast temperature ramp and rapid heat transfer between the heating block and the PCR mixture. This idea led to the development of the miniaturized PCR devices. The miniaturized PCR devices when compared with the conventional PCR device has the advantage of small thermal mass, require small sample volumes, low cost, low power consumption and integration [6, 7]. The miniaturized PCR chips are classified into two major groups: the stationary chamber and the continuous flow (flow-through) PCR. The stationary chamber PCR is further divided into three groups: single chamber [8, 9], multi-chamber [10, 11] and the virtual reaction chamber (VRC) [12-14]. The continuous flow PCR device has five divisions: on-chip serpentine rectangular [15-23], the circular arrangement of three temperature regions (spiral channel-base [6, 24], single circular channel [25] and cylindrical capillary channel [26-29]), the single straight capillary oscillatory flow [30, 31], Multi-chamber based CFPCR [32-34] and the convection-driven CFPCR [35]. In the stationary chamber PCR, the sample is injected into the well and the chip is heated and cooled through specific thermal cycling temperatures of denaturation, annealing and extension. The process of heating and cooling of the entire chip generates an unwanted inertial effect due to thermal mass. In a continuous-flow PCR, the sample moves through

the fixed temperature zones to achieve the required thermal cycling. In this case only the sample is heated and cooled rather than the entire chip. This consumes lesser energy and allows rapid thermal cycling of the sample.

Kopp *et al* [16] designed the first serpentine CFPCR. The design of a serpentine CFPCR device is such that the experience a temperature gradient by passing through the extension region before annealing takes place. The effect is that melted single-strand DNA (ssDNA) samples will directly form double-stranded DNA (dsDNA) without amplification. To circumvent this problem, the sample should pass the extension region without experiencing the extension temperature. For this to be achievable, the channel will need to be very narrow at that region of extension temperature so as to increase the velocity of the PCR sample. Another problem with the serpentine original design is the thermal cross talk between the three thermal blocks used for the three temperature regions. Since the design is based on temperature gradient, the middle thermal block can be eliminated and two remaining block can be placed at both ends of the chip and a temperature gradient can be achieved at the middle part of the channel. Crews *et al* [36] carried out an experimental work on the above design. Computational fluid dynamics (CFD) makes it possible for such device to be extensively studied and achieved optimization before manufacturing. This paper presents a preliminary study to establish the pressure required by the chip to carry out amplification and the velocity profile of the sample and reagent in the channels. These will be achieved by using commercial software ANSYS CFX for simulations.

2. Computation

The PCR was modelled in single phase laminar flow using Computational Fluid Dynamics (CFD) techniques. The Navier Stokes equations were solved and discretized in first order advection with convergence limit of 1E-5 for the residuals. The SIMPLE algorithm was used in coupling the pressure and velocity terms. The working fluid is water and assumed pre-heated to 95^{o}C.

2.1 Model Formulation

The continuity equation is given in equation (1), the momentum equation is described by equation (2), and the energy equation is of the form in equation (3).

$$\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \boldsymbol{U}) = 0 \tag{1}$$

$$\rho \left(\frac{\partial \boldsymbol{U}}{\partial t} + \boldsymbol{U} \cdot \nabla \boldsymbol{U} \right) = -\nabla P + \rho \boldsymbol{g} \tag{2}$$

$$\rho \left(\frac{\partial h}{\partial t} + \nabla \cdot (h \boldsymbol{U}) \right) = \nabla \cdot (k \nabla \mathbf{T}) + S_h \tag{3}$$

$$\mathbf{h} = \int_{Tref}^{T} \mathbf{C_p} \, \mathbf{dT} \tag{4}$$

Where h is the sensible heat. S_h is a source term and k is the molecular conductivity and T_{ref}=95^{o}C.

2.2 Model Setup

A tetra-mesh grid from 500K to 3M nodes was used to avoid mesh independence on the solution regime. The geometry is boxed within 17×22×0.005mm. This chip model geometry has similar design with the one reported by Crews et al [35]. The narrow and wide channels widths were 110 and 650µm and spaced 450µm apart with 50µm deep. The mass flow rate is 550µg/s and the wall boundaries are adiabatic and at no slip conditions. The bottom plate is at 65^{o}C while the inlet condition is at 95^{o}C.

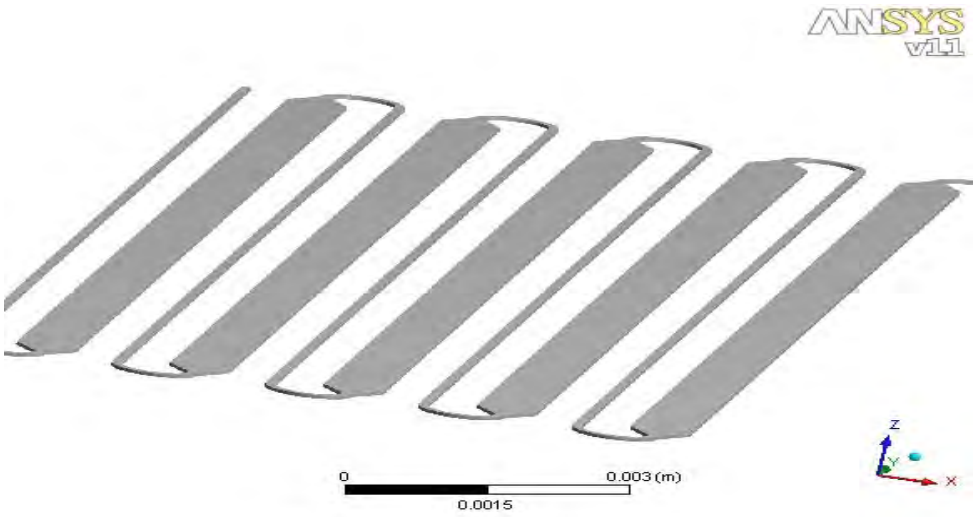
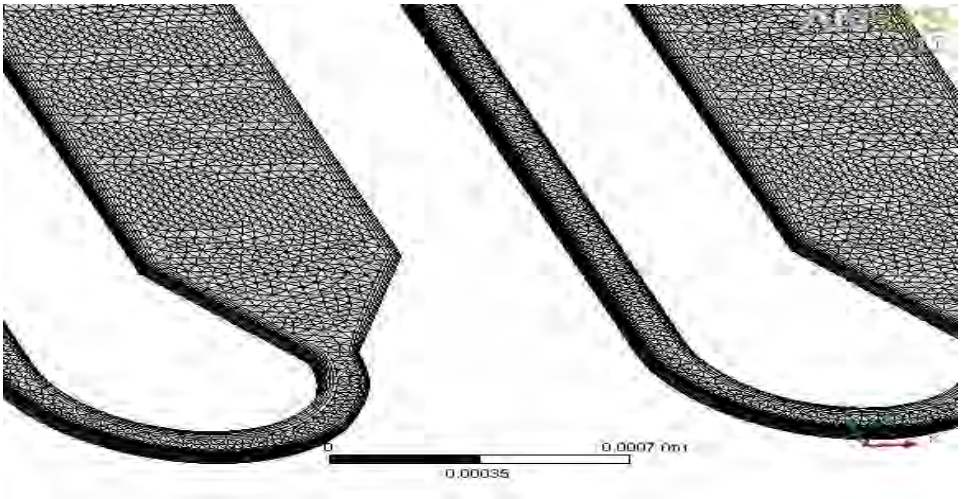


Fig. 1. Geometry of Modelled Channel.



(a)

Fig. 2. Volume Mesh of the model.

3. Results

The temperature and pressure distributions along the channel are at channel mid-depth position of 25μm. The velocity distribution shown is a cross sectional of the model channels and 12mm from the base of the modelled device. The temperature, pressure and velocity distribution are shown in figures 3, 4 and 5. The pressure drops along the wider and narrow channels are shown in figure 6 and figure 7. The amplification temperature along the wider channel is shown in figure 8.

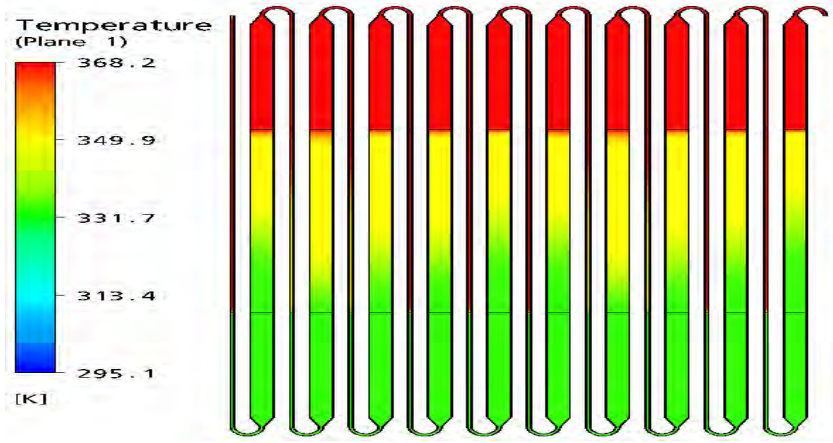


Fig. 3. Temperature distribution at mid-channel depth

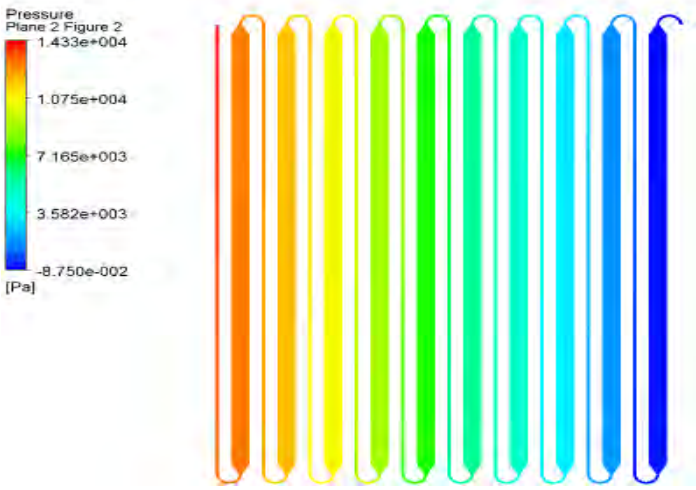


Fig. 4. Pressure distribution at mid channel depth

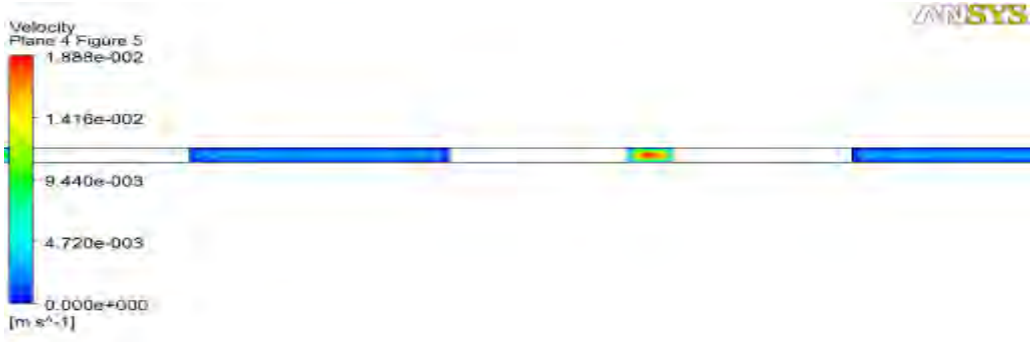


Fig. 5. Horizontal cross sectional view of Velocity profile at mid channel depth

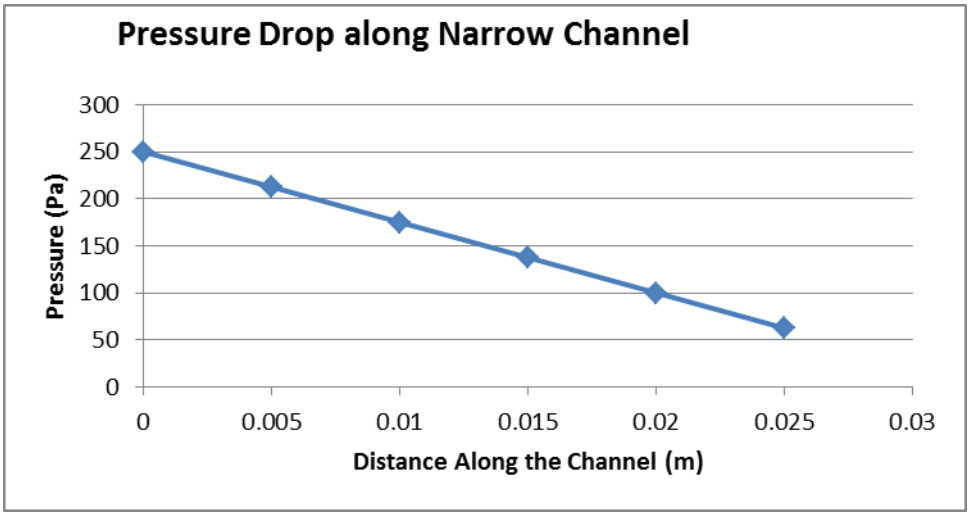


Fig. 6. Pressure Drop along the Narrow channel

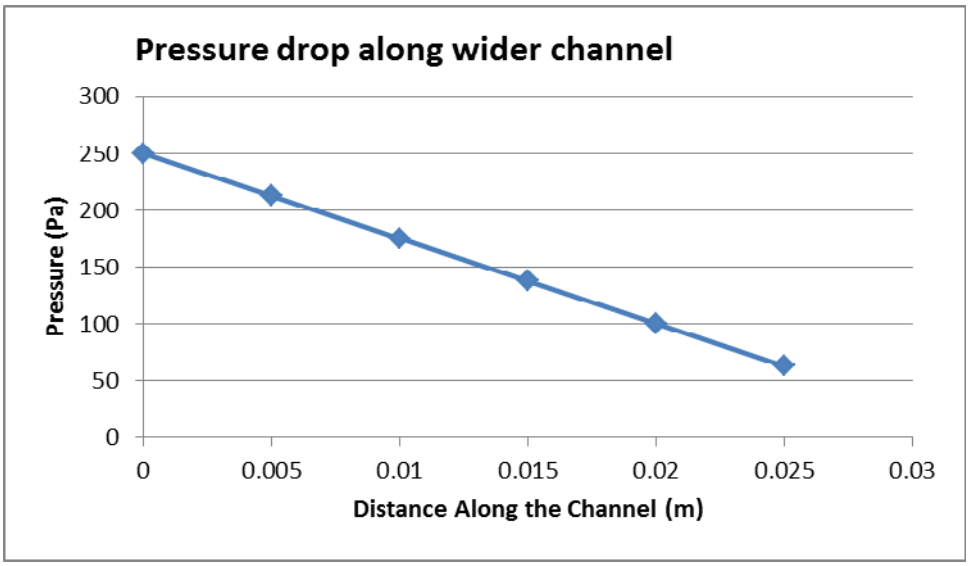


Fig. 7. Pressure Drop along the Wide channels

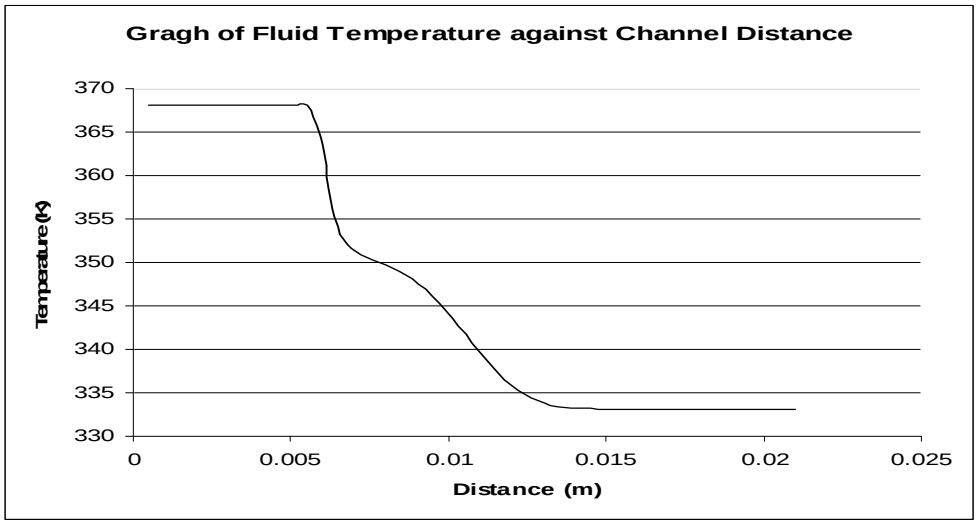


Fig. 8. PCR amplification process on the wider channel

4. Discussion

4.1 Temperature Distribution

For a DNA amplification to take place in a PCR, the PCR sample must experience three distinct temperatures of denaturation, annealing and extension. In this model two temperatures of 95^0C and 60^0C was used and placed at the top and bottom of the model. However, due to temperature gradient, another region of extension is also visible. These three distinct temperature regions enable amplification to take place (figure 3). Fig. 8 shows the actual amplification process of PCR. The temperature at mid-point along the graph achieved the extension temperature of 72^0C and is same as the one proposed by Crew *at al*.

4.2 Pressure Drop

Most literature pays much attention to the temperature distribution in the PCR chamber. One major problem facing the serpentine CFPCR is the fact that double stranded DNA is form without amplification. This drawback, which has now been solved with fabrication of a narrow and wide channel by Crews *et al* 2008 still have some concern on whether the pressure drop across the channel will have negligible effect on the portability of the device. While embracing the new development as the best way out for CFPCR, the result obtained from simulation for the pressure distribution is too huge to be ignored. The pressure drop in the 10 cycle channel from 14300.7 to 85.2 Pa figure 4, 6 and 7. The rate at which pressure drops is even more alarming; 58.35 Pa/mm and 7.5 Pa/mm for a narrow and wide chamber respectively. At this rate for a 30 cycle PCR pressure is about 42.6 KPa. It therefore means that incorporating other analytical steps and devices to form a single chip will be a difficult task.

4.3 Velocity Profile

The model was designed to increase the velocity of PCR sample at the narrow the narrow chamber while the wide chamber will have a very low sample velocity to enable the full formation of dsDNA, such that the sample will move so fast that it will not experience the 72 ^{0}C long enough to cause the formation of a double stranded DNA. The simulated result (figure 5) shows that the velocity across the depth of the channel is higher at the narrow channels and lower at the wide channels.

5. Conclusion

ANSYS CFX was used to model and simulate the temperature, pressure and velocity in a serpentine CFPCR. The model consists of narrow and wide chambers and used only two temperature heaters for the denaturation and annealing, while the extension was achieved through temperature gradient. The simulated result shows that the new design achieved increase in velocity at the narrow chamber, thereby solving the problem faced by similar previous design by ensuring that the narrow chamber did not experience extension temperature. Despite this achievement, the pressure drop across the 10 PCR cycle model is about 14.2 KPa, amounting to 42.6 KPa for a 30 cycle model. This pressure drop is too high and will therefore affect the full integration of this device as μTAS. The multi-chamber CFPCR with an integrated pneumatic micropumps and valves offers a better approach of performing PCR.

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