# DNA Amplification by PCR using Low cost, Programmable Microwave Heating

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## ABSTRACT

This paper presents a low power, low cost programmable micro-liter capacity PCR thermo-cycler for portable/Point of Care (POC) genetic analysis. The proposed thermo-cycler is based on non-contact, local heating of PCR chamber via microwave radiation. A microstrip transmission line is used to deliver approximately 0.7 Watts of power to the chamber, which has 4 µL capacity and is embedded in a polycarbonate substrate. Preliminary results suggest that a temperature of 92 °C can be achieved when the chamber is filled with water. Temperature profile programmability is realized by varying the input amplitude of the microwave signal and/or its frequency. Electrical and thermal modeling of the device and measurements from a fabricated prototype are provided.

***Keywords***: *microwave heating, point of care (POC) genetic analysis, Polymerase chain reaction, DNA amplification.*

## 1 INTRODUCTION

Polymerase Chain Reaction (PCR) is a central tool in modern biology and medical research to replicate DNA. Millions of copies of specific DNA fragments are created by cycling through three temperatures multiple times in the presence of DNA polymerase, DNA oligonucleotides (primers-DNA building blocks), and probes (required to specify the amplified region). Applications range from genetic analysis, forensics, evolutionary biology, to cancer diagnosis. Conventional PCR instruments normally achieve a heating/cooling rate of 1-2°C/s in the temperature range relevant for PCR, thus a complete PCR analysis would require 1-2 hours [1], mainly due to the high thermal mass of typical thermo-cycling systems. Recent advances in PCR micro-fluidic technologies have created miniaturized, lower cost, and highly integrated devices that facilitate DNA amplification at much faster rates as a result of smaller thermal capacity and larger heat transfer rate between the PCR sample and temperature controlled element [2]. A recent publication reported resistive IC-based micro-heating elements using poly-silicon encapsulated in glass, and coupled to polymeric micro-fluidic channels [2]. However, these micro-heating elements typically consume enormous amount of power with high voltage requirements. Micro-heating elements also provide fixed heating profiles depending on the physical structure of the heater. Other techniques involve the use of IR light, and flow-through temperature zones [3]. Sa far, the majority of existing research has focused on miniaturized devices with fixed heating profile that are individually optimized for certain specimen or targets.

Compared to the previous approaches, microwave heating delivers heat directly to the fluid with up to 95% efficiency using less power, while providing a uniform heating profile. The main advantage for microwave-based heating is that the heating rate is controllable by changing the frequency/amplitude of the microwave signal. Recent studies have also eliminated prior concerns that microwave radiation can destroy DNA samples, and found appreciable enzyme activity after tens of cycles of microwave power [4]. As Microwave PCR gathers trend, researchers have demonstrated nearly 100% yield efficiency even in very dilute sample concentration [5]. However, existing technologies are limited due to high volume or high frequency operation. While high volume PCR requires a magnetic stirrer for uniform heat distribution [5], high frequency PCR (> 10GHz) often suffers from substrate losses, which results in less power delivered to the sample [6]. The objective of this work is to investigate miniaturized, microwave-based thermo-cyclers and to asses their potential for parallel DNA amplification.

## 2 MODELING OF MICROWAVE-BASED THERMOCYCLER

### 2.1 Electrical Modeling

The microwave energy is coupled to the micro-fluidic chamber using a microstrip transmission line as shown in Fig. 1. Tuning stubs are added to increase the coupling efficiency by minimizing reflections from the micro-fluidic chamber which acts as a load to the transmission line. The load is modeled as a parallel combination of a resistor and capacitor, where the resistance and capacitance are given as

$$R_s = \frac{d}{2\pi f \varepsilon''(T,S)\varepsilon_0 A} \qquad (1)$$

$$C = \frac{\varepsilon\ (T,S)\varepsilon_0 A}{d} \qquad (2)$$

where,
d = depth of microfluidic chamber in mm,
f = frequency of operation in Hz,
A = cross-section of micro-fluidic chamber in $mm^2$,
$\varepsilon_o$= permittivity of free space in F/m,
ε(T,S) = relative permittivity of the fluid.

Water was selected as the working medium, since DNA amplification employs mainly water based solutions. Furthermore, water is best suited for microwave heating since it has high permittivity and loss tangent, facilitating coupling of electromagnetic waves. The permittivity of water is a strong function of temperature and frequency and can be modeled by the following equation [7];

$$\varepsilon(T,S) = \frac{\varepsilon_s(T,S) - \varepsilon_1(T,S)}{1 + i\dfrac{v}{v_1(T,S)}} + \frac{\varepsilon_1(T,S) - \varepsilon_\infty(T,S)}{1 + i\dfrac{v}{v_2(T,S)}} + \varepsilon_\infty(T,S) - i\frac{\sigma(T,S)}{2\pi\varepsilon_o v} \qquad (3)$$

where,
ε(T,S) = dielectric constant of water as a function of Temperature (T) and salinity (S), $\varepsilon_1$(T,S) = intermediate frequency dielectric constant, which is the dielectric constant of water recorded at a frequency between low (kHz) and very high ( THz) frequency ranges, $\nu_1$(T,S) = first Debye relaxation frequency (GHz) , $\nu_2$(T,S) = second Debye relaxation frequency (GHz) , ε(T,S) = dielectric constant at infinite frequencies (THz), $\varepsilon_s$(T,S) = static (zero frequency) dielectric constant (units), σ(T,S) = conductivity of water (in S/m), T: temperature (in °C), and S: salinity (in parts per trillion).

The variable frequency load impedance is inserted in a 2D electromagnetic simulation environment (Momentum™), and used for the design of the transmission lines and tuning stubs to guarantee maximum coupling of microwave energy to the micro-fluidic chamber for all temperatures of interest.

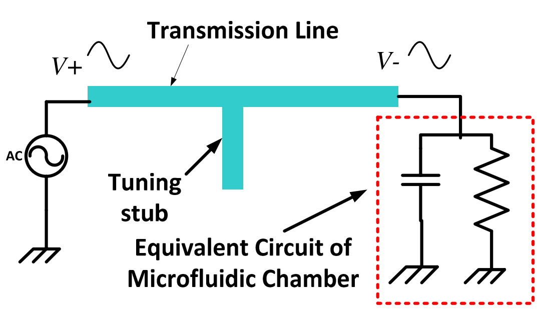


Fig. 1: Thermo-cycler configuration; a matched transmission line coupling energy to the micro-fluidic chamber.

## 2.2 Thermal Modeling

For miniaturized transmission lines, the level of microwave power than can be delivered to the DNA amplification chambers is limited. To estimate if the power level achievable under given geometrical constrains is sufficient for generating the required heating in a reasonable amount of time, the device was simulated employing transient heat transfer carried out with a commercial finite element modeling software (Abaqus). The modeling is also necessary in order to predict the optimum distance between two adjacent PCR chambers, which must have an independent temperature profile. This is critical when designing arrays of thermocyclers for parallel DNA amplification.

For simulations, a simplified model of the device was used. The model, shown in Fig. 2 takes into account the polycarbonate substrate and the solution in the PCR amplification chamber. Transmission and ground lines were not included at this stage of the modeling. The thermal properties of the solution were approximated as being those of water (thermal conductivity k=0.6 W/mK, specific heat c=4184 J/kgK and density, ρ=1000 $kg/m^3$), which is a reasonable assumption since the amplification takes place in a water based medium. The thermal properties of the polycarbonate were taken as k=0.2

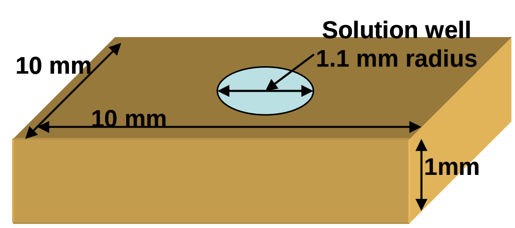


Fig. 2. Geometry used for finite element modeling.



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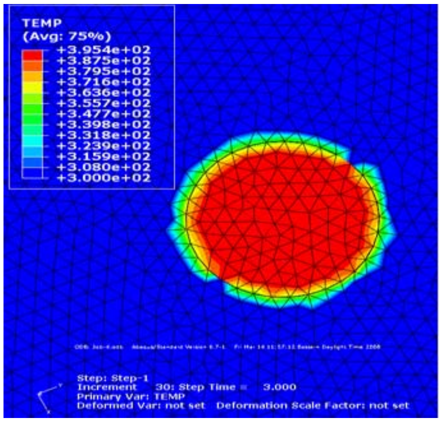


Fig. 3. Temperature distribution in in well and substrate, 3 seconds after microwave power application (finite element modeling carried out with Abaqus).

W/mk, c=1200 J/kgK and ρ=1300 kg/m$^3$. The geometry of the PCR well was cylindrical, with a radius of 1.1 mm, while the size of the substrate was taken as 1cm x 1cm square with a thickness of 1 mm. Convection losses due to natural convection were considered on both sides of the device. The heat transfer coefficient for natural convection was approximated as 10 W/mK. Radiation losses were neglected, though they will be considered in future modeling. However, although the temperature of the well is expected to reach ~95°C, in applications the well is covered on both sides with a copper line (transmission and ground lines) that have very low emissivity coefficient (ε~0.1), hence radiation losses are rather negligible. For initial simulations, the applied power was estimated as 0.45W. The calculated temperature profile after 3 seconds of applied power calculated is shown in Fig. 3. As can be seen in this figure, for the applied power density the well temperature rises to ~94°C (typical values required for DNA amplification) within 3 seconds. The temperature inside the well is uniform and the heating of the substrate is negligible. At present efforts are being made to model a more accurate representation of the device for both, heating and cooling conditions.

## 3 DEVICE FABRICATION

### 3.1 Substrate Selection

The selection of a suitable substrate for the construction of a microfluidic structure is of vital importance as it should satisfy the thermal, chemical, electrical and machining requirements. An ideal material would provide thermal insulation during the heating phase and dissipation during the cooling phase. Most of the plastics in use are thermal insulators. Glass offers robustness, transparency and chemical inertness, but is a poor conductor of heat. PDMS is a low cost, transparent with flexible texture, but suffers from hydrophobicity leading to fluid flow problems in microfluidic channels. Also, it has a relatively high loss tangent that makes it quite lossy for high frequency power transmission. For proof-of-concept demonstration, polycarbonate was chosen as substrate as it can be easily machined, is chemically inert, optically transparent and with good permittivity and low loss tangent at high frequency. The thickness of the polycarbonate substrate was 1.0mm. The microfluidic chamber drilled out had a capacity of 4 μL.

### 3.2 Transmission Line Design

A microstrip transmission line design is chosen for delivering microwave power from an external generator and power amplifier to the micro-fluidic chamber load as shown in Fig. 4. The microstrip structure is implemented using a 50 μm copper line on polycarbonate substrate. The matching stub is added to avoid microwave power reflected back to the input source and allow maximum power delivery to the load.

The transmission line structure is analyzed using a network analyzer. The resulting reflection is measured using S-parameter representation. An $S_{11}$ response below -10 dB is indicative of over 90% power transmission to the load with minimal power returned or reflected back.

## 4 EXPERIMENTAL RESULTS

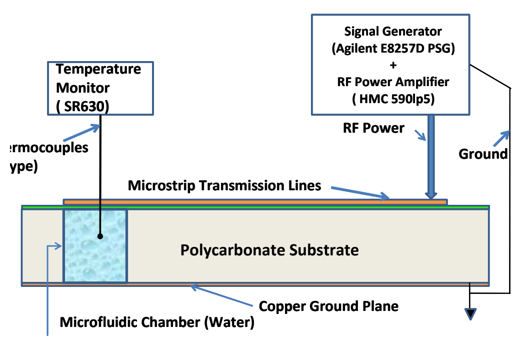


Fig. 4 : Design of Microstrip Transmission line (Width of the transmission line is 2.5 mm, overall length is 12.5 mm, matching stub is located 5.45 mm from left side and has a length of 2.9mm).

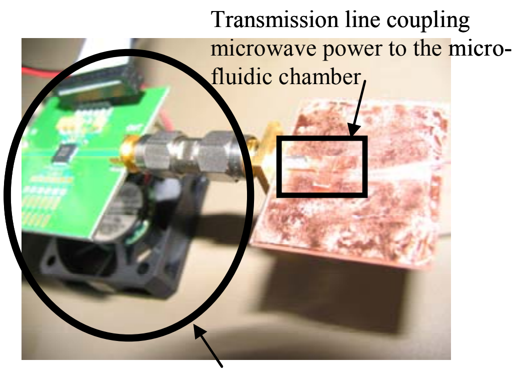


Fig. 5: Experimental prototype of the microwave based thermo-cycler

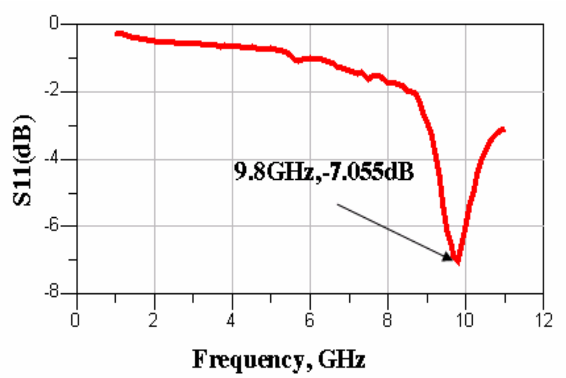


Fig. 6: $S_{11}$-response showing the reflected microwave signal from a transmission line thermocycler loaded with microfluidic chamber

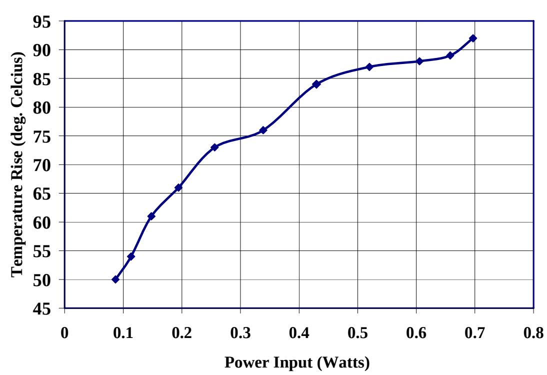


Fig. 7: Measured temperature variation in the fluid as a result of varying the level of RF input power

The experimental prototype is shown in Fig. 5. The microwave signal is supplied by a CW signal generator (Agilent E8257D) and amplified with an external power amplifier (Hittie HMC 590LP5). The power delivered to the PCR chamber is 0.7Watts at 9.8GHz. However only 80% of the power is coupled to the microfluidic chamber. The reflection characterized by S-parameter measurements as shown in Fig. 6. Preliminary temperature measurements were carried out employing an E-type, 25 µm diameter thermocouple wire that was is inserted in the microfluidic chamber. Figure 7 presents the change in steady-state temperature versus applied microwave power. These results indicate that a temperature of 92°C can be achieved at 0.7W applied power. However, metallic thermocouples are not immune to microwave radiation and at present fiber optic thermometry is being implemented to perform more accurate temperature measurements.

## 5 CONCLUSION

Modeling, design, and fabrication of a low power, cost-effective, POC thermocycler based on programmable microwave heating is reported. Preliminary results indicates that 0.7W microwave power delivered to a water filled, 4 µL well causes up to 67°C increase in temperature above ambient.. The main advantage of current device is that the temperature profile can be programmed by changing the input microwave amplitude and/or frequency. This can ultimately be used in multi-chamber microfluidic arrays to carry out versatile PCR reactions simultaneously without compromising on yield efficiency, reagents or sample quantity.

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