Structural optimization for heat detection of DNA thermosequencing platform using finite element analysis

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For the past three decades, Sanger's method has been the primary DNA sequencing technology; however, inherent limitations in cost and complexity have limited its usage in personalized medicine and ecological studies. A new technology called "thermosequencing" can potentially reduce both the cost and complexity of DNA sequencing by using a microfluidic platform [Esfandypour, Pease, and Davis, *J. Vac. Sci. Technol. B* **26**, 661 (2008)]. To optimize the efficiency of the technology, finite element analysis was used to model the thermosequencing system by simulating the DNA incorporation reaction series and the resulting product concentration and heat production. Different models of the thermosequencing platform were created to simulate the effects of the materials surrounding the system, to optimize the geometry of the system, and to concentrate reaction heat into specific regions for detection in the real system. The resulting concentrations of reaction products were used to calibrate the reaction speed and to design the heat sensors in the thermosequencing technology. We recommend a modified gated structure for the microfluidic detection platform by using control valves and show how this new platform could dramatically improve the detection efficiency. © 2008 American Institute of Physics. [DOI: [10.1063/1.2901138](https://doi.org/10.1063/1.2901138)]

I. INTRODUCTION

Sanger's DNA sequencing method has been the dominant large-scale DNA sequencing technique for the past three decades.^{1,2} Though its cost has decreased by three orders of magnitude since Sanger sequencing's invention, the technique remains impractical for nonresearch oriented and personalized DNA sequencing due to inherent limitations in cost, throughput, read length, and enzymatic complexity.³⁻⁶

A novel DNA sequencing-by-synthesis method, which we call "thermosequencing," circumvents Sanger sequencing's limitations by directly measuring the heat produced in DNA synthesis for an inexpensive, fast, and label-free DNA sequencing technology.⁷⁻¹¹ This paper describes modeling results for the DNA reaction speed, heat detection, and geometric optimization of the thermosequencing system.

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FIG. 1. Schematic of thermosequencing platform.

In the thermosequencing platform (Fig. 1), a magnetic microbead is coated with single-stranded DNA and rests at the bottom of a well attached to a microfluidic channel etched in polydimethylsiloxane (PDMS). The following formula represents the reaction that occurs in the well for thermosequencing:

Here dNTP represents a nucleotide, which is transported by the microfluidic channel to the well and incorporated into a growing strand of DNA. The reaction releases heat as a product, which is ultimately detected by a thermometer on the bottom of the well to determine the existence of reaction with the specific dNTP molecule injected.¹² The DNA sequence is determined by injecting one type of nucleotide at a time into the channel and determining the existence of reaction for that nucleotide type.

Currently, the throughput and read length of the state-of-the-art sequencing techniques are at around $5\text{--}6 \times 10^6$ base pairs (bp) and 500–800 bases per second (bps) for Sanger technology, $20\text{--}40 \times 10^6$ bp and 100–500 bps for Roche-454 pyrosequencing technology, 1×10^9 bp and 25 bps for ABI/Agencourt technology, and 1.2×10^9 bp and 35–50 bps for Illumina-Solexa's sequencing technology. The thermosequencing technique would, potentially, be able to provide 1.3×10^9 bp and 500–800 bps.

It has been shown by Torres *et al.*¹³ that microscale thermistor sensors can detect a $500 \mu\text{K}$ change in temperature with a signal-to-noise ratio of 6. The goal of this study then is to prove the feasibility of such a thermistor sensor to measure directly the heat generated by the nucleotide incorporation reaction and to optimize the geometry of the thermosequencing platform for high temperature change sensitivity.

A. Reactions chain of DNA thermosequencing

Though the overall dNTP incorporation reaction is shown above, the actual incorporation reaction consists of seven distinct reactions, with each reaction's products as reactants for the following reaction:

TABLE I. Reaction series constants.

Reaction	Forward constant	Reverse constant
1	$1.2 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$	0.06 s^{-1}
2	$1 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$	50 s^{-1}
3	50 s^{-1}	3 s^{-1}
4	150 s^{-1}	37.5 s^{-1}
5	15 s^{-1}	15 s^{-1}
6	1150 s^{-1}	$5 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$
7	$1.2 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$	0.06 s^{-1}

Each reaction has the forward and backward rate constants¹⁰ found in Table I.

Because the incorporation of dNTP into elongating DNA is a series of reactions, all reactions except the first and last were modeled using COMSOL multiphysics, with heat production modeled in the last reaction. Modeling the series of reactions allows for accuracy in the time scale of reaction and in calculating the rate-limiting reaction on the bead. The first and last reactions were not included in the simulations because we assumed that polymerase and single-stranded (ss) DNA do not separate during synthesis of the elongating chain.

B. Finite element analysis equations and considerations

The ruling application modes for thermosequencing modeling are diffusion and conduction. The diffusion equation is

$$\delta_{\text{is}} \frac{\partial c}{\partial t} + \nabla \cdot (-D \nabla c) = R,$$

where δ_{is} is the time-scaling coefficient, D is the diffusivity of the material, c is the concentration of the material, and R is the reaction rate. For the thermosequencing models, δ_{is} is set to 1, the diffusivity of nucleotide is set to $3.68 \times 10^{-10} \text{ m}^2/\text{s}$ (Ref. 13 but is set to $5 \times 10^{-11} \text{ m}^2/\text{s}$ for ease of computation, and the diffusivity of the product inorganic phosphate (PPi) is set to $1 \times 10^{-11} \text{ m}^2/\text{s}$.¹⁴ For these models, there is no convective momentum transport simulated because we assume there is an initial concentration of the nucleotide in the channel for these models at 3 mM.

FIG. 2. The time-dependent concentrations of dNTP and PPI at the well bottom are shown for the 2D simulation. The dNTP concentration asymptotically increases to equilibrate with the 3 mM dNTP concentration in the channel. The maximum PPI concentration at the well bottom is 0.1407 mM (time scale is in seconds).

The heat conduction equation also uses the heat equation and is

$$\delta_{ts} \rho C_p \frac{\partial T}{\partial t} - \nabla \cdot (k \nabla T) = Q,$$

where, again, δ_{ts} is the time-scaling coefficient, ρ is the density of the material, C_p is the heat capacitance of the material, and k is the thermal conductivity of the material. In this stage of thermosequencing, we assume that polydimethylsiloxane (PDMS) maintains perfect insulation and set ρ , C_p , and k to the density, heat capacitance, and thermal conductivity of water at 25 °C, or 997 kg/m³, 4184 J/kg K, and 0.58 W/m K, respectively. In the equation, Q is the heat source and is coupled to the reaction rate of PPI production, which releases 12.3 kcal/mol of PPI produced.¹⁵

II. FINITE ELEMENT SIMULATION RESULTS OF REACTION SPECIES

In these COMSOL simulations, we modeled the thermosequencing system by simulating the DNA incorporation reaction series and the resulting product concentration and heat production, in both two and three dimensions, to confirm the simulation results of concentration and temperature change between the two models for greater precision and, if the concentration and temperature change results are congruent, to allow for the future use of two-dimensional (2D) simulations in place of three-dimensional (3D) simulations for ease of simulation.

A. 2D simulation of dNTP and PPI concentrations

1. 2D structure

In the 2D model, the height and width of the well are both modeled to be 5 μm . The height of the fluid channel is 5 μm . The bead has a radius of 1.4 μm , and the DNA is estimated to be 0.5 μm long, uniformly resting on the surface of the bead. Nucleotide is assumed to be in the channel at the beginning of simulation and diffuses down into the well as the simulation progresses.

2. 2D reaction species

As the dNTP diffuses into the DNA wells, it reacts with the DNA_n on the bead surface and produces PPI. Figure 2 details the concentrations of dNTP and PPI at the bottom of the well as time varies from 0 to 1.25 s dNTP is shown to diffuse down into the well as its concentration

FIG. 3. Control channel schematics for the double (top left) and single (bottom left) control line systems. In both cases, pressurization of the control channels induces expansion for mass and thermal insulation of the well. The double-control line system for a 100- μm -wide channel is shown at right.

gradually increases to reduce the concentration gradient. The maximum concentration of PPi in the system is found at the bottom of the well at 0.3 s to be 0.18 mM. After 0.3 s, however, PPi's concentration gradient forces its diffusion into the microfluidic channel.

B. 3D simulation of PPi concentration

1. 3D structures

To create the 3D model of the thermosequencing system, it was shown^{10,16} that three separate geometries needed to be modeled to optimize thermodynamic detection. The first 3D geometry models an open channel with one well and bead to ensure correspondence with the 2D model. In this geometry, all lengths, except depth of the well, were preserved from the 2D model. The depth of the well is modeled to be 5 μm .

To provide thermal insulation for the heat produced by the dNTP incorporation, a set of gated fluid channels orthogonal to the microfluidic channel (Fig. 3, top left) were designed so that, on command, the microfluidic channel would be sealed by the expanding control channels to prevent heat and species diffusion. The idea of adding microfluidic gates to control an existing flow channel is introduced in^{5,8,9} for mechanical occlusion of materials transport in large, 100- μm -wide channels. Here we propose to use them for both mechanical occlusion of dNTP and PPi and thermal insulation in the 5- μm -wide microfluidic channel in thermosequencing.

To further reduce the heat diffusion outside the well, a second insulation system was designed (Fig. 3, bottom left), with one main control channel directly on top of the well which could seal the well directly.

2. 3D reaction species results

Figure 4 shows the PPi concentrations in all three 3D models. In the open-channel 3D model, the PPi concentration profile at 0.42 s, with 0.12 mM at the bottom of the well, is only 68% of the maximum concentration simulated by the 2D model. However, like the 2D model, PPi still diffuses down the microfluidic channel and does not stay localized to the reaction well.

In the 3D model with two control lines, the PPi concentration profile had a maximum concentration of 0.10 mM at the bottom of the well. However, because of the insular properties of the control channels, the PPi did not diffuse down the microfluidic channel. Instead, the PPi concentration was confined to a steady-state value of ~ 0.06 mM at the local reaction region.

Finally, in the 3D model with one control line, the maximum PPi concentration at the bottom of the well at 0.5 s is ~ 0.1 mM. In this model, however, all of the PPi produced remains in the well, so that the steady-state concentration of PPi in the well is ~ 0.08 mM.

FIG. 4. Time-dependent PPI concentrations are plotted for the open-channel, two-control-line and one-control-line models of thermosequencing. The one-control-line PPI concentration begins to increase more quickly than the others because it is assumed that, when the control line begins pressurizing, the expansion will convectively drive dNTP into the well for faster reaction.

III. FINITE ELEMENT SIMULATION RESULTS OF HEAT PRODUCTION AS A FUNCTION OF WELL SIZE

A. Temperature change results for a 2D open-channel system

Figure 5 shows the temperature change at the bottom of the well for the 2D model. At 0.5 s, the reaction has mostly completed and the temperature change at the well is 390 μK . However, because the thermistor heat sensor has a sensitivity of 500 μK , it is desired for the temperature change to be at least 1 mK for robustness of detection.

B. Temperature change results for the 3D open-channel system

Figure 6 describes the temperature change in the 3D models. In the open-channel model, the first-order temperature change was observed with maximum temperature change at 484 μK at 0.5 s. This result is slightly higher than the 2D temperature change at 0.5 s, which was calculated to be around 390 μK . The difference in temperature change between the two models may be attributed to the 2D model's approximation of the depth of the channel and well.

The temperature change for the 3D open-channel system is due to the rapid diffusion of heat into the microfluidic channel, which heated up the fluid in the microfluidic channel along with the fluid inside the well. Heat diffused too quickly throughout the system to localize the temperature change to the bottom of the well, where the temperature change detection will occur. Thermal insulation would be needed to prevent heat diffusion into the channel and allow for greater detection accuracy and lower thermal noise in neighboring wells.

FIG. 5. The time-dependent temperature change at the well bottom is shown for the 2D simulation. The temperature change is 390 μK at 0.5 s.

C. Modified gated microfluidic channel architecture

1. Double channel on structure sides

The effect of rapid heat diffusion along the microfluidic channel of the thermosequencing platform was investigated to optimize the detection platform; as a result, the previously described control channel structures were devised. Assuming that the control channels on either side of the well in the fluid channel can insulate the microfluidic channel, the maximum temperature change becomes 750 μK , as seen in Fig. 6.

2. Single top channel structure

Under the single control line structure, Fig. 7 shows that the temperature change in the bottom of the well increased substantially to around 1670 μK , which would substantially reduce temperature detection error as well as thermally insulate the heat from one well to neighboring wells.

D. Optimization of size of system

Subsequent modeling focused on the size of the structure to optimize the accuracy and range of heat detection. To accomplish this, two axis-symmetric models of the thermosequencing platform were simulated, where one model was volumetrically 1000 times larger than the other.

To determine the optimum size and geometry of the DNA reactor for heat sensing, wells and channels of two different sizes were compared for maximum temperature change from the heat of reaction. The two models are 2D and defined axis symmetrically with axis through the top of the bead. The first model's dimensions are equal to those of the 3D model with two control lines. Figure 8 shows that the reaction reached steady state in around 2 s and the maximum temperature change was 800 μK .

FIG. 6. Comparison of the temperature change for the open-channel, two-control-line and one-control-line systems from 0 to 0.5 s. It is apparent that the insulated systems have higher temperature change due to the decrease in the thermal conductivity and the decreased heat absorption by the fluid surrounding the reaction.

The second model was a $10\times$ scale version of the second model. Both the bead and the well dimensions have increased 10-fold, while the DNA_n concentration on the surface of the bead was assumed to be constant from the first model because we assume that the number of DNA strands on the bead depends on the surface area of the bead. During the course of simulation at 2 s, the reaction did not reach steady state in temperature change or reaction, with only a part of the DNA_n reacted. In addition, Fig. 8 shows that the maximum temperature change in the well at 2 s was found to be 21 μK , almost 40 times less temperature change than the $1\times$ model. Therefore, to preserve shorter sequencing times and to increase the temperature change in the well for sensing accuracy, the size of the system should be set to the first model's size, with dimensions $5\times 5\times 5\ \mu\text{m}$ wells and microfluidic channels with $5\times 5\ \mu\text{m}$ cross-sectional area.

E. Effect of bead's material

Thus far all models created have neglected the thermal effects of the magnetic beads, which are M270 Dynabeads™ from Invitrogen Corporation. Based on material properties provided by Invitrogen, the M270 has the following thermal properties:

- 1) density: $1600\ \text{kg/m}^3$,
- 2) specific heat capacity: $1.059\ \text{kJ}/(\text{kg}\cdot\text{K})$,
- 3) thermal conductivity: $1.51\ \text{W}/(\text{m}\cdot\text{K})$.

Using the single control line scheme described above with the Dynabead thermal properties, the temperature change in the system at 0.5 s is depicted in Fig. 7. The maximum temperature change of the well system, assuming that the bead is the M270 Dynabead, is 1620 μK , which is only 50 μK below the temperature change calculated for the system without the bead.

To ensure this estimate is appropriate, a model was created for the system where the magnetic bead was simulated to be pure iron (specific heat capacity: $0.450\ \text{kJ}/(\text{kg}\cdot\text{K})$). In this model, the maximum temperature change was found to be 1585 μK . Therefore, it can be concluded that the presence of the bead does not absorb an error-causing amount of heat from the dNTP reaction.

FIG. 7. The temperature change in the one-control channel system with 2.8 μm Invitrogen Dynabead™ at 0.5 s. The density, heat capacity, and thermal conductivity characteristics are calculated and simulated. The difference in temperature change between this model and the standard one-control channel system is around 90 μK .

IV. DISCUSSION

From the simulation results, it is apparent that the maximum concentration of PPi at the bottom of the well is on the order of ~ 0.1 mM. Because the initial concentration of DNA_n on the bead is set to 1 mM and is the limiting reagent, the amount of PPi produced in the system is dependent on the amount of DNA_n initially on the bead.

In addition, to concentrate the PPi and heat production to the bottom of the well and to reduce chemical crosstalk between neighboring wells, the volume of the reactor must be reduced and the microfluidic channel must be sealed off at the well.^{5,8,9} In the 3D simulations of the system with control lines implemented, we show that expanding control lines localize PPi concentration and, assuming perfect insulating material, drastically concentrate heat into the sensing region of the well, causing a 1670 μK change in temperature change.

The simulation results also show that while the reaction occurs very quickly, the time-limiting factor in PPi production is the time of diffusion of dNTP. From the models, it seems that it takes around 0.5 s for dNTP to fully diffuse down into the well for reaction.

V. CONCLUSION

FEM simulations show that the current system can be improved. To increase heat production for detection robustness, the amount of reaction, and consequently the amount of DNA on the

FIG. 8. A comparison of the temperature change in sequencing platforms of two different sizes. The $1\times$ size system (top) is the aforementioned two-control channel system. The $10\times$ size system (bottom) is the same two-control channel system increased in volume by 1000. The limits on the diffusive flow of dNTP are seen as the $10\times$ size system is actively heating up the system at 2 s while the size system reaches thermal and reaction steady state.

bead, must be increased. To prevent thermal and chemical crosstalk between neighboring wells, insulation in the form of control channels must be installed to limit the volume of reaction. Finally, the time of diffusion of dNTP into the system is limiting the total time of reaction and PPi production; therefore, a redesign of the system is required for faster convective dNTP flow.

With these encouraging simulation results of thermosequencing, other challenges and aspects of thermosequencing must be considered in future work. Fabrication of the sensor, PDMS wells, and control channels at the $5\ \mu\text{m}$ scale could pose significant problems. The thermal insulation properties of PDMS must be considered in fabrication as well. Finally, noise and common-mode rejection issues must be resolved for sensor robustness and detection accuracy.

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