

Microfluidic Systems for DNA Sequencing

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Abstract:

DNA sequencing is one of the most important tools in biological studies and provides a key insight into living organisms. The recent development of pyrosequencing has proven itself to be a much simpler and faster means for sequencing than traditional methods. It is currently limited, however, by its ability to produce only short read lengths. In order to make the process more efficient, we integrated the pyrosequencing technique into a microfluidic system to increase the reaction speed and also reduce the required sample size. We did this by creating a new technique that implements continuous liquid flow through microfabricated channels in silicon. We matched the dimensions of the channels to that of the capillary tubes to minimize the dead volume loss, and in doing so, hoped to achieve longer read lengths. The ability to achieve longer read lengths from the pyrosequencing technique would simplify the genome assembly process.

Introduction:

DNA sequencing has changed the dynamics of biomedical and medical studies. As more research is being conducted on the genetic make up of organisms, there is an increasing need to reduce the time, complexity, and cost of sequencing. The recent development of pyrosequencing has proven itself to be a much simpler and faster means for sequencing than traditional methods [1]. In this process, visible light is generated that is proportional to the number of nucleotides added. When polymerase adds a correct nucleotide to its base pair, the system releases a pyrophosphate molecule which is subsequently converted to ATP by the enzyme ATP sulfurylase. The ATP powers the enzyme luciferase, an enzyme commonly found in fireflies, to oxidize luciferin and produce light. This method eliminates many of the items needed for dideoxy sequencing such as labeled primers, labeled nucleotides, and gel electrophoresis.

Traditionally pyrosequencing has been conducted by separately adding the four nucleotides to DNA in a

test tube. Light is generated when a correct nucleotide is added, and the extra nucleotides are degraded by the enzyme apyrase. This system however, is not 100% effective and leads to a build up of by-products and residual chemicals. In addition, the system becomes increasingly dilute as more nucleotides are added. These two factors limit the length of DNA that can be sequenced, and the short read lengths make the genome assembly process more difficult.

In order to make the process more efficient, we integrated the pyrosequencing technique into a microfluidic chip. To address the problem of short read lengths, we designed the chip for continuous liquid flow, so that we could introduce fresh chemicals and flush out residual chemicals. We matched the dimensions of the chip to that of the capillary tube to eliminate dead volume areas where there could be a residual chemical build-up. By doing this, we hoped to both reduce the required sample size for sequencing as well as achieve longer read lengths.

Procedure:

We designed the microfluidic channel to have the same volume capacity as a 1/32 inch diameter capillary tube.

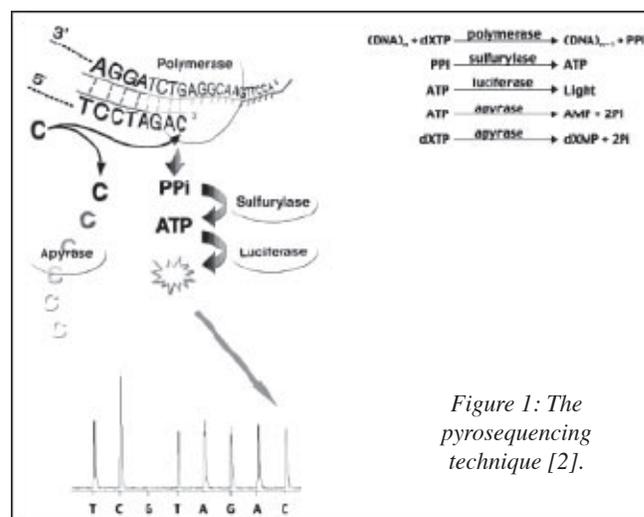


Figure 1: The pyrosequencing technique [2].

Microfluidic Chip

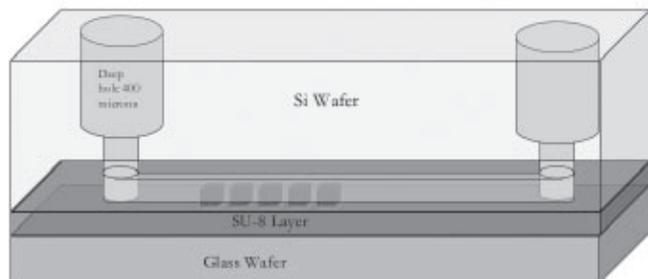


Figure 2: The complete microfluidic chip. Capillary tubes are placed directly into the holes etched in the silicon wafer.

In order to complete the chip, we had to design four mask layers which required both frontside and backside alignment, as well as optimize processes for two different substrates: silicon and SU-8. First, we etched $150\ \mu\text{m}$ diameter holes $100\ \mu\text{m}$ deep into a silicon wafer. We then etched a $50\ \mu\text{m}$ deep channel on top of the two $150\ \mu\text{m}$ diameter holes. We flipped the wafer over and etched large $900\ \mu\text{m}$ diameter holes $400\ \mu\text{m}$ deep to meet the small holes and complete a through-hole etch.

Afterwards, we coated a glass wafer with $50\ \mu\text{m}$ of the negative resist, SU-8, and proceeded to expose a single row of $50 \times 50\ \mu\text{m}$ wells into the SU-8. These wells served as resting areas for the DNA coated beads. These beads are introduced into the wells by drying a solution of beads over the wells. The glass wafer is then visually aligned to the silicon wafer etched with the channels, and clamped in place with a jig.

Results and Discussion:

Since we wanted to use a continuous flow system in the chip to flush out residual chemicals, we had to eliminate dead volume areas where there could be a nucleotide build up. We did this by designing the chip so that the capillary tube could be directly inserted into the chip without a connector. The diameter of the big hole was thus the same as the outer diameter of the capillary, and the diameter of the small hole was the same as the inside diameter of the capillary. The volume capacity of the capillary was equal to that of the channel.

Another unique aspect of the microfluidic chip was the use of SU-8 to form wells for the DNA coated beads. SU-8 is a hydrophobic, epoxy based photoresist. By

forming wells down to the glass we formed hydrophilic pockets in SU-8 where the beads could sit. We tested our hypothesis by drying beads on a surface and then viewing the beads with an optical microscope. We saw a high efficiency filling of the wells, indicating an affinity of the beads for the wells.

Future Work:

The next step for the project is to test the microfluidic chip by introducing liquids through a syringe and measuring the light producing reaction.

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References:

- [1] Ronaghi, M. Pyrosequencing sheds light on DNA sequencing. *Genome Research*. 2001 Jan;11(1):3-11.
- [2] Nordfors, L. Large Scale Genotyping of Single Nucleotide Polymorphisms by Pyrosequencing and Validation Against the 5' Nuclease (Taqman) Assay. *Human Mutation*. 2002. 19:395-401.

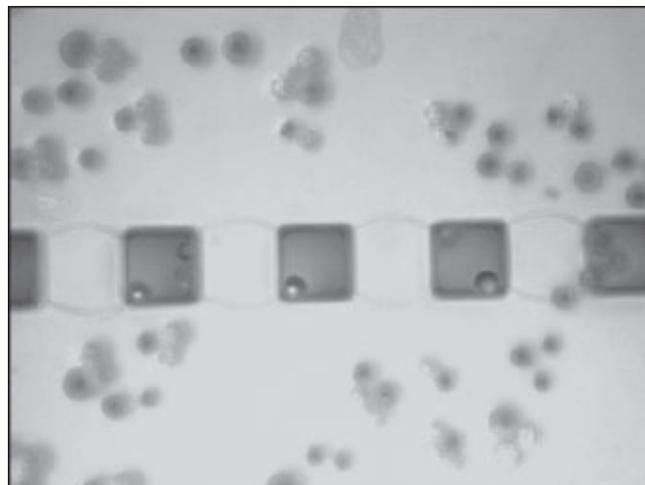


Figure 3: High efficiency beads filling of SU-8 wells.