Miniaturizing DNA Sequencing Technology: Designing Microfluidic Channels for Performing Chemistry on Beads

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Abstract:

The availability of several complete organismal genomic sequences, made possible by drastic reductions in sequencing cost in concert with advances in sequencing technology, has revolutionized the nature of biological and biomedical research. Yet for this rapid progress to continue, and to enable the genomes of countless more organisms to be sequenced cheaply and efficiently, a new approach to DNA sequencing that exponentially decreases its current costs must be developed.

Pyrosequencing is one such novel approach that is based on the detection of visible light generated via an enzymatic reaction cascade occurring in response to the successful incorporation of nucleotides during DNA elongation. In this project, we design, fabricate and package a miniaturized microfluidic version of the pyrosequencing process as a prototype of a potentially more cost-effective and rapid method of DNA sequencing.

The progression of microfluidic channels is etched in a silicon wafer; the wafer is then bonded to a glass wafer with holes drilled at positions corresponding to the channel inlet/outlet ports; finally, the enclosed channels are connected to the macroscopic world of reagent supplies and tested for successful microfluidic capabilities. A successful prototype will demonstrate the potential for miniaturizing DNA sequencing to a lab-on-a-chip scale.

Introduction:

In February of 2004, the National Human Genome Research Institute issued a request for grant applications "to develop novel technologies that will enable extremely low-cost genomic DNA sequencing." [1] Presently, a mammalian-sized genome can be sequenced for $10 to $50 million. The ultimate goal of the NHGRI is to reduce costs by at least four orders of magnitude, to produce the $1000 mammalian genome. Substantial fundamental research is needed for such a major advance, and it is anticipated that the realization of this goal may take up to a decade to achieve [1].

Of the many alternative approaches to the dideoxy chain termination technology most commonly employed to sequence DNA today, pyrosequencing is a particularly promising method [2]. The pyrosequencing process (Figure 1) begins with the release of pyrophosphate when polymerase successfully adds a correct nucleotide to a growing primer strand hybridized to a template DNA molecule. This pyrophosphate is then converted to ATP by ATP sulfurylase. ATP then provides the energy for luciferase to oxidize luciferin, a reaction that generates light and occurs naturally in fireflies.

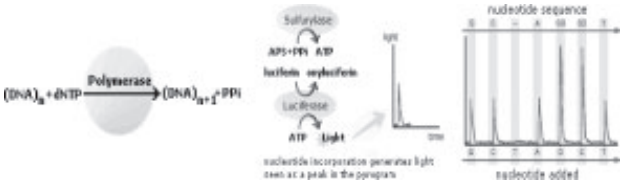

Figure 1: The pyrosequencing process miniaturized in this work [3].

The pyrosequencing technique, then, is based on the detection of light released when the correct nucleotide is incorporated into each successive position on the growing DNA strand. As the four types of nucleotides are added to the reaction mixture one type at a time, the sequence of the template can be determined.

In this project, we develop a prototype for a miniaturized pyrosequencer by designing a series of microfluidic channels etched in silicon and sealed with a glass cover. These microfluidic channels must secure in a stationary location certain beads with attached single-stranded DNA templates, permit all of the requisite enzymes and nucleotides to flow, allow one type of nucleotide to be washed away before the introduction of another, enable detection of generated light, and facilitate connections to the macroscopic world.

Procedure:

We designed a series of microfluidic channels consisting of a main channel with a protruding pillar to contain the 30 μm-diameter DNA beads in single file at a downstream point, and six separate inlet channels into the main channel for flow of enzymes, wash, and the four types of nucleotides (Figure 2). The channel inlets were widely separated in anticipation of the macroscopic size of capillary connections to the microfluidic channels. We created a mask with four motifs of the design that varied the diameter of the main channel from 30-60 μm and the pillar from 10-40 μm in order to test the effectiveness of flow through channels of different widths. We transferred the mask design to silicon test wafers using standard photolithographic methods and

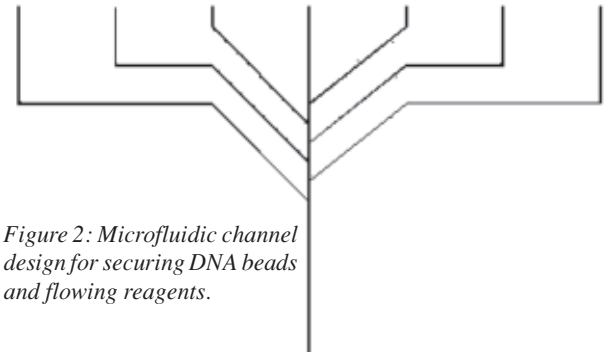

Figure 2: Microfluidic channel design for securing DNA beads and flowing reagents.

etched the design using plasma etching. Inlet and outlet holes were manually drilled into glass wafers using a drill press with a 1.1 mm diamond tip, and then these cover glass wafers were anodically bonded to the etched silicon wafers. Using Upchurch microfluidic-to-capillary connections, we packaged the wafers for reagent flow and prototype testing.

Results and Discussion:

SEM and optical microscope pictures of key elements of the microfluidic pyrosequencing prototype show their successful fabrication (Figures 3-4). When the channels were connected to macroscopic connections, deionized water could be flowed through the channels smoothly, with little back-pressure and minimal obstruction by particles or bubbles, even through the narrowest pillar region of the main channel. A significant amount of fluid, however, is diverted up the side channels if pressure is not applied at their inlet ports. If a light signal can be detected when pyrosequencing reagents are flowed through the channels, this will be the first prototype for a miniaturized pyrosequencer.

Future Work:

The next steps will be to run colored fluid through the channels for better flow visualization, then DNA beads and the rest of the pyrosequencing reagents to test the prototype's pyrosequencing capabilities. Further work on this project includes trying different types of microfluidic-to-macroscopic connections based on other connector prototypes in the literature, designing inlet channels that will prevent contamination of one type of nucleotide by another, and automating enzyme distribution methods.

Acknowledgments:

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References:

[1] http://grants.nih.gov/grants/guide/rfa-files/RFA-HG-04-003.html.

[2] Ronaghi, M. 2001. Pyrosequencing sheds light on DNA sequencing. Cold Spring Harbor Laboratory Press. www.genome.org/cgi/doi/10.1101/gr.150601.

[3] Graphic adapted from http://ww.pyrosequencing.com/pages/technology.html.

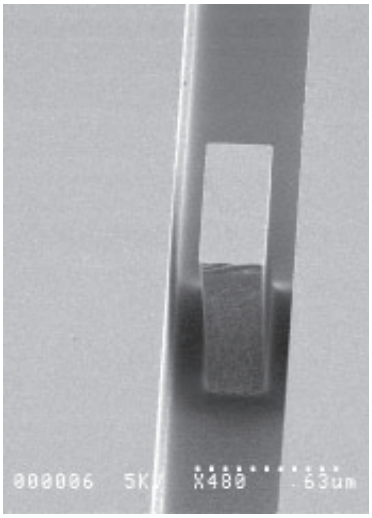

Figure 3: SEM picture of pillar region of main channel.

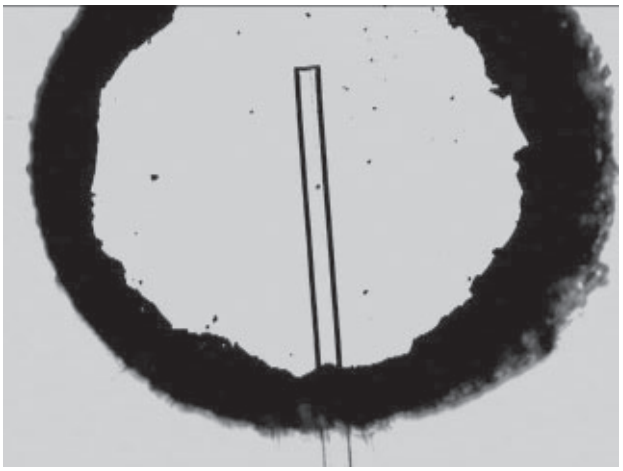

Figure 4: Optical microscope picture of inlet hole over etched silicon channel.