Microfabrication of a Parallel-Array DNA Pyrosequencing Chip

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Abstract:

DNA sequencing is essential to genomics and must be performed efficiently. Pyrosequencing is a new innovation that confirms a correct nucleotide addition by emitting light through enzymatic reactions. The objective is to fabricate a parallel-array pyrosequencer on a chip. The obstacle is to bring a camera sufficiently close to the microwells where the sequencing takes place so it can capture emitted light while eliminating crosstalk errors between adjacent wells. We have tested several designs for achieving this objective. If one succeeds, we will have developed an enabling technology for a scaled down DNA sequencer.

Introduction:

Developing a low-cost genomic DNA sequencer has become an urgent necessity in order for genomics to progress. The NHGRI has been issuing grant requests for developing such low-cost alternatives [1]. The current cost of sequencing a mammalian genome is $10 million. Pyrosequencing shows promise to lower this to $10,000.

Unlike Sanger sequencing, which utilizes gel electrophoresis, pyrosequencing uses enzymatic reactions to detect correct nucleotide additions. This procedure change reduces bulky DNA sequencing equipment to a silicon chip. As detailed in Figure 1, the first pyrosequencing step is the release of a pyrophosphate (PPi) from correct nucleotide incorporation [2]. The PPi is converted to ATP using the enzyme ATP-sulfurylase, which then provides energy for the firefly enzyme luciferase to oxidize luciferin and emit light. This light is then recorded by a camera.

Current technology brings the camera to within 500 μm of the reaction wells, by resting the camera on top of a glass wafer. This limits well size and pitch to no less than 500 μm; if the size or pitch was any smaller, crosstalk error would occur between the chip wells and camera pixels. The objective here is to bring the camera to 10-50 μm of the wells. This will allow experimentation with smaller well sizes and pitches, and result in a large increase in well density and parallel processing.

Experimentation and Results:

Four different channel patterns and four different well patterns were designed to test functionality of the parallel-array aspect of the chip. At the micrometer scale, fluid flow is purely laminar. Thus, it is necessary to fabricate something to spread a 1 mm flow of fluid coming from the inlet channel over the 6 mm wide atrium where the wells reside. A series of pillars (designs 1 and 4) and branches (designs 2 and 3)

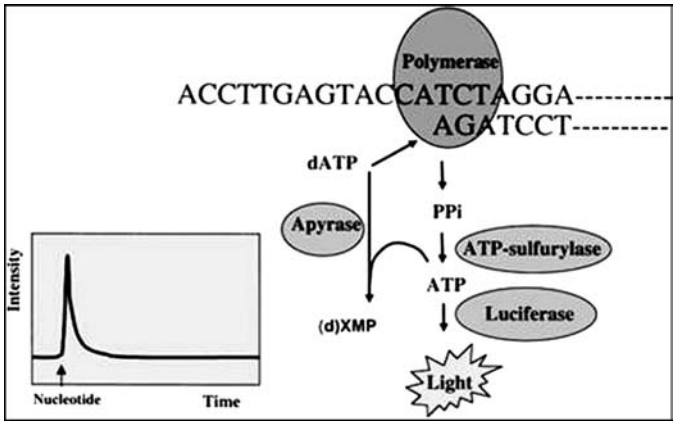

Figure 1: Pyrosequencing process. Light indicates correct nucleotide incorporation.

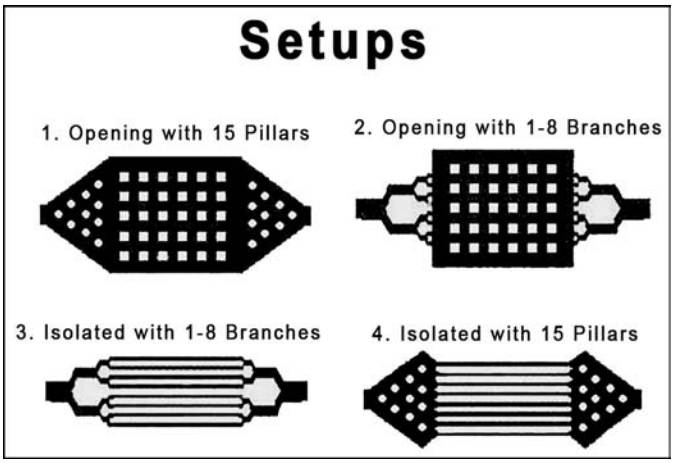

Figure 2: 4 channel/well designs. Pillars/wells exaggerated.

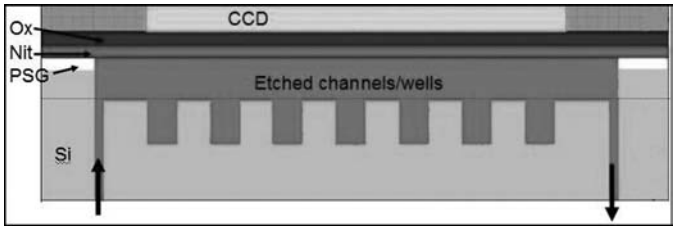

Figure 3: Cutaway depiction of initial experiment.

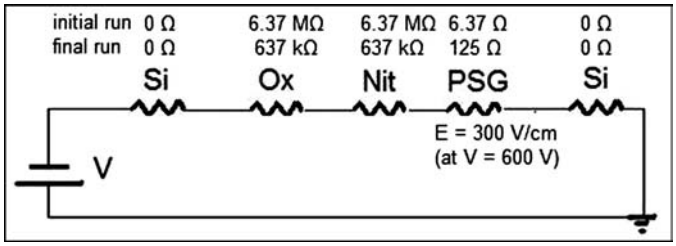

Figure 4: Electrical representation of bonding process, with initial/final resistances.

accomplish this (see Figure 2). Isolating rows of wells is another method, which was implemented in designs 3 and 4.

The first procedure attempted involved etching wells and channels, and drilling inlet/outlet holes into one silicon wafer. On a second sacrificial silicon wafer, 5000 Å layers of SiO_2, Si_3N_4, and PSG were deposited in succession. This procedure is illustrated in Figure 3. The wafers were anodically bonded through the PSG, but the bonding failed. This prompted an investigation into the bonding process to determine why failure occurred. By deriving the electrical model in Figure 4, it was determined that bonding failed because the electric field across the PSG was too small, at 5 V/cm. The oxide and nitride layers were reduced to 500 Å and the PSG layer was increased to 10000 Å. In addition, the PSG layer was etched to reduce its area from 78.5 cm^2 (the area of the 4″ Si wafer) to 8 cm^2 (the 500 μm surrounding each channel). This decreased the oxide and nitride resistances and increased the PSG resistance, since layer resistance is $R = \rho t/A$, ρ being resistivity, t being thickness, and A being area. This increased the electric field over the PSG to 300 V/cm. However, anodic bonding still failed, leading to the conclusion that even a field of 300 V/cm was insufficient for anodic bonding.

The next method involved anodic bonding between silicon and Pyrex® 7740 wafers. This has been shown to succeed, and calculations show the electric field produced across the 500 μm glass wafer is 10000 V/cm, in contrast to the earlier 300 V/cm. This method required etching wells, channels, and drilling inlet/outlet holes into one silicon wafer. The next steps were to bond to glass and reduce glass thickness, which required HF etching. A 49% concentrated HF solution etches Pyrex® 7740 at 8.5 μm/min [3].

A 50 μm thinned Pyrex® wafer was bonded to the back of a double-polished silicon wafer, and a normal glass wafer was bonded to the front, before HF etching. This protected the drilled holes and the inside of the front glass wafer from HF, and served to mark when the stack should be removed from the HF solution. When the back Pyrex® wafer disappeared, it indicated the front Pyrex® wafer was 50 μm thick and the stack should be removed. This method succeeded in leaving a thin glass membrane of 15 μm.

Future Work:

The successfully fabricated chips need testing. The first test will be to run water at varying rates and observe if the glass membrane will withstand the pressure generated. These results will be compared with a standard glass wafer bonded to silicon. If these chips perform satisfactorily, then initial pyrosequencing data will be collected, and compared to the current standard as well. If these tests return satisfactory results, we will have accomplished our objective to create a more efficient DNA sequencer.

Acknowledgements:

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References:

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[2] Ronaghi, M. 2001. "Pyrosequencing Sheds Light on DNA Sequencing". Cold Spring Harbor Laboratory Press.

[3] Diepold, T. and E. Obermeier. 1996. "Smoothing of Ultrasonically Drilled Holes in Borosilicate Glass by Wet Chemical Etching". IOP Publishing Ltd.