Electrowetting for DNA Sequencing on Chip

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Abstract:

Digital microfluidics is a technique that is based on controlled manipulation of unit-sized microdroplets. One of the methods for microdroplet actuation is electrowetting, which involves using an electric field to modify the wetting behavior of a nano-liter-sized liquid droplet in contact with an insulated electrode. Electrowetting allows for controlled dispensing, transport, mixing, and splitting of large numbers of these droplets across electrode arrays. We extend the use of the electrowetting device to DNA sequencing on a silicon wafer.

In our study, we designed and built arrays of electrodes, wired to a series of control pads programmed to run the reaction of pyrosequencing of DNA. Upon incorporation of a nucleotide, a pyrophosphate molecule is released, which will be converted to ATP and, in a cascade enzymatic reaction, produce visible light which will be detected at a site away from the reagents. By utilizing the electrowetting device to perform miniaturized pyrosequencing of DNA, the development of a more reliable, higher-automated sequencing technology is in sight.

Introduction:

Electrowetting is the enabling technology behind miniaturized lab-on-chip systems, which is changing the way experimentation is being done today. Electrowetting involves using linear arrays of electrodes for manipulation of unit-sized liquid droplets. These microdroplets, which act as solution-phase reaction chambers, are transported, mixed, split, reacted, or analyzed in a discrete manner using a standard set of basic, programmable instructions [1, 2]. These basic instructions are used to create a lab-on-chip environment for miniaturized experimentation. In Figure 1, the two-plate electrowetting setup is shown.

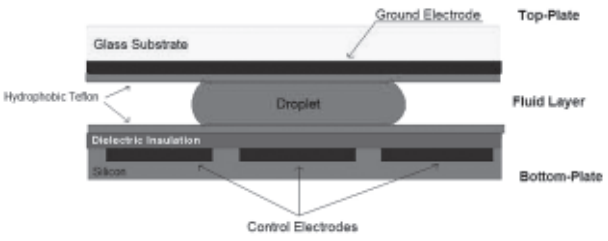

Figure 1: The electrowetting setup: A side view of digital microfluidics.

Electrowetting works much the same way as traditional analysis systems, only with much smaller volumes and much greater automation. Continuous fixed-flow microfluidics is a simpler alternative to electrowetting, but offer little in terms of reconfigurability and scalability [2]. Fixed flow channels also require large volumes of liquid for priming, which is not very cost-effective.

Work is being done to test the applications of electrowetting on wider ranges of established chemistries and protocols by scaling down to the nanoliter droplet format. In our study, we extend the use of electrowetting technology to pyrosequencing of DNA on a silicon wafer. Pyrosequencing is a DNA sequencing technique involving detection of released pyrophosphate and visible light resulting from DNA synthesis [3]. First, a single-stranded DNA template reacts with a nucleotide and DNA polymerase to produce pyrophosphate. In a cascade of enzymatic reactions, ATP is generated, which drives the reaction of luciferase with luciferin to produce visible light proportional to the amount of ATP present. Each nucleotide

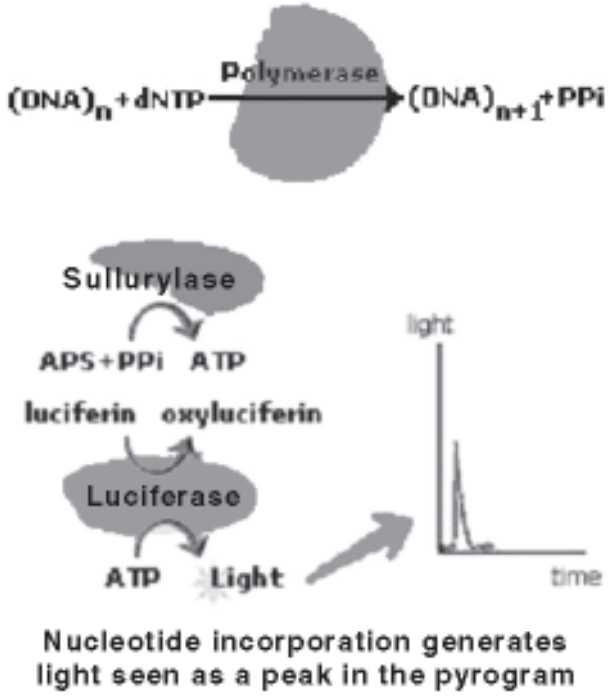

Figure 2: Cascade enzymatic reaction of pyrosequencing.

is added one at a time, and since the nucleotide added is known, the sequence of the DNA can be determined. This process shown in Figure 2, although it sounds simple, is quite expensive and cumbersome.

Our research group wishes to miniaturize the process, reducing the amount of sample and reagents needed. Miniaturization of pyrosequencing using electrowetting will reduce costs and increase automation, both crucial to large scale genetic testing.

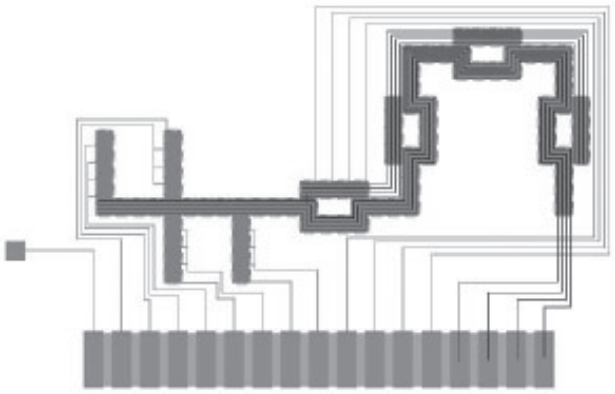

Figure 3: Our four-layered design of the electrowetting device.

Procedure:

Using the CAD program L-Edit, we designed our four-layered electrowetting device as shown in Figure 3. First a layer of oxide was grown on our silicon wafers. We then exposed and developed the buried wire layer on the surface of the oxide. After etching, we sputtered titanium metal on each wafer. We then baked in the oven at 650°C for 30 mins which created a titanium silicide layer and a titanium nitride layer.

After stripping the nitride layer, we deposited a thin layer of low temperature oxide. Next we exposed and developed the via-hole layer onto our wafers, etched holes down to the buried wire layer and sputtered aluminum metal on each wafer. We exposed and developed our electrode layer onto the surface of the aluminum and etched the surrounding aluminum away to form our patterned electrode arrays. We then deposited one more layer of LTO and etched holes down to our control pads. Finally we coated the wafer with a thin layer of Teflon®.

For the top plate, we coated a glass wafer with a thin layer of indium tin oxide, which we annealed to our substrate at 340°C for 10 minutes. Next we coated the wafer with Teflon®. Finally we brought the bottom plate and top plate together with spacers and tested the device for movement of droplets across the electrode arrays.

Results and Discussion:

We have completed the fabrication of the electrowetting chip. In Figure 4 is a TEM image of two interlocking electrodes from our completed wafer. We tested oscillation of droplets across electrodes, and we successfully moved liquid droplets across the four-phase transport hub. We believe this lab-on-chip for pyrosequencing of DNA using electrowetting shows great promise. Our study suggests that our device may help to miniaturize the process of DNA sequencing to lower costs and increase throughput and automation. We have yet to test the dispensing, splitting and merging structures on the chip, and our research group is still working on a means of detection of visible light. If a light signal can be detected when pyrosequencing reagents are reacted on our electrowetting chip, this will be the first step towards a successfully miniaturized pyrosequencer.

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References:

[1] Duke University, http://www.ee.duke.edu/Research/microfluidics/.

[2] Vijay Sriinivasan, Vamsee K. Pamula and R.B. Fair, An integrated digital microfluidic lab-on-a-chip for clinical diagnostics on human physiological fluids, Lab on a Chip, 2004, 4, 1-11.

[3] Mostafa Ronaghi, Pyrosequencing sheds light on DNA sequencing. Genome Research, 2001, 11, 3-11.

[4] Graphic adapted from www.pyrosequencing.com/pages/technology.html.

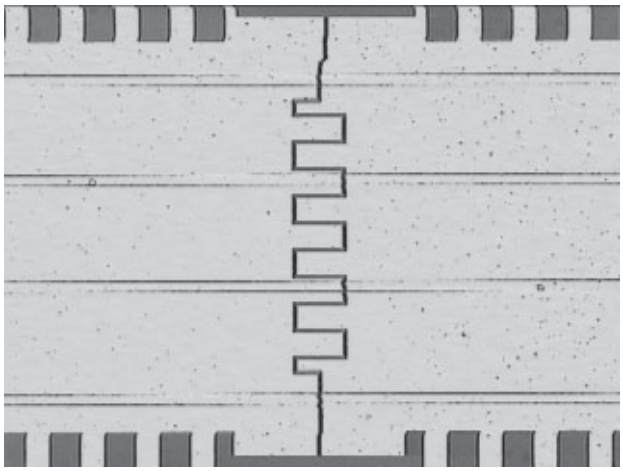

Figure 4: TEM image of two interlocking electrodes.