# A Novel Receptacle for Gene Sequencing

H. Esfandyarpour[1,2], R.W. Davis[1]

[1] Stanford Genome Technology Center
855 California Ave., Palo Alto, CA, 94304, USA
[2] Center of Integrated Systems, Department of Electrical Engineering, Stanford University
CISX B103, Stanford, CA, 94305, USA
hesaam@stanford.edu

## ABSTRACT

In this paper we present a new platform for gene sequencing based on microfabricated channels by multilayer soft lithography with coated iron beads. The encapsulation of the reaction of DNA polymerization in picoliter-sized wells provides for excellent isolation and control for detection and prevents cross-talk amongst neighbor reactors which is one of the most limitations for higher integration of the current technologies. Through external magnetic field the beads can be allocated with better accuracy. The proposed system is useful for a number of other bio-species detection and sorting templates. This paper illustrates the design and experimental results of the primary template as well as different advantages and potentials of this platform in the world of DNA sequencing and genetics.

***Keywords***: DNA sequencing, microfluidic channel, PDMS, magnetic beads, valve-controllable.

## INTRODUCTION

The revolution that the Sanger method of DNA sequencing [1] and its subsequent array automation [2] has made in biology and genetics was followed with the sequencing of the human genome and the completion of the Human Genome Project [3, 4]. The Sanger dideoxy DNA sequencing method has been the most commonly used method for DNA sequencing thus far, particularly in large-scale genomic sequencing, but inherent limitations of this state-of-the-art technology such as cost, throughput and read length, and enzymatic complexity make it impractical to deliver the current needs of DNA sequencing for clinical applications, disease detection and discovery, genomics studies, diagnostics and drug delivery, and personalized medicine.

The Human Genome Project was essentially accomplished after a reduction in the cost of DNA sequencing by three orders of magnitude. It is desired to reduce the cost by another three orders of magnitude to enable profiling of the individual genome. To achieve this goal, a highly integrated platform with high throughput and reliability will be needed.

In 2005, Nature published an article by Margulies et al. entitled "Genome Sequencing in Microfabricated high-density picolitre reactors" [7]. The paper describes embedding beads coated with DNA in wells etched into the end of a fiber optic slide. Each well serves as a reaction chamber for the pyrosequencing method, a sequencing-by-synthesis technique [5, 8, 9, 12, 18, 19]. They are making an instrument for this research built as a part of 454 Life Science Corp. which has 1.6 millions picoliter wells. However, not all of these wells can function as a reactor. (In one of the latest 454 machine as the-state-of-the-art technology, the maximum number of wells that could count as good spots with reliable results has not exceeded ~250,000 from 1.6,000,000 wells). One of the main issues that could limit the technology for higher integration is the cross-talk between neighbor wells and each well and its bead can affect on the adjacent ones. Another reason is that the platform could suffer from the effect of previous run cycles due to washing and flow issues e.g. since high pressure fluidic injection is not practical.

Here we introduce an effective and useful microfluidic platform for DNA sequencing, especially for sequencing-by-synthesis methods (e.g. pyrosequencing). Additionally, the proposed picoliter micofluidic system is essentially useful for a number of other bio-species detections and sorting templates [6, 15, 16, 17]. This platform is based on a PDMS gate-controlled channel with a number of wells incubated on its wall, which leads to encapsulation of the beads in these picoliter wells as sensing volumes and can significantly improve the signal to noise ratio of detection by preventing cross-talk between adjacent wells. The encapsulation of bead or reaction in the picoliter-sized vessels provides for excellent isolation and control on the detection-based systems by preventing cross-talk among neighbor reactors.

In addition, by using micro-sized iron beads instead of conventional sepharose or glass beads, we can gain more flexibility for allocation control, movement or holding the beads by an external magnetic force and improving the injection and washing phases, and helping to use higher pressure flow in the channel. The electromagnetic field can be applied by the use of a simple cubic magnetic bar located on the right side of the system close the wells (~5

mm)) or using a MEMS magnetic actuator which gives higher control and accuracy (in high-density production).

In conclusion, the current state-of-the-art technology could lead to a much higher sensitivity in real time.

## METHODS AND MATERIALS

The proposed micofluidic platform is based on the valve-controllable (or gate-controllable) microfluidic system made of polydimethylsiloxane (PDMS) [11, 13, 14, and 16] which contains micrometer-sized iron beads (e.g. 2.8 um-in-diameter M270 DYNAL Bead [10]). Fig.1 shows the design schematic of the initial platform design for the proposed system. The red lines in Fig.1 shows the main flow line and the green lines are the control lines as a layer perpendicular in direction and on top of the flow lines (red) with about 20 µm thin PDMS membrane between these two layers which prevent mixing but the elastic property of PDMS makes it possible to close the flow line b of PDMS, which allows closure of the flow line by pressurizing the control valve [11, 14, 16]. The primary channel, which has been made in Stanford Microfluidic Foundry and used in the experiments of the next section, has a width of 100µm and height of about 10µm (theoretically it was designed and fabricated for 10µm but in practice, the physical channel height is usually smaller than the theoretical value, due to upside pressure and some process variations. As it is shown in the design, this channel includes two inlets and one outlet plus both controlled with a top layer control valve [11, 13, and 16]; In addition, this system has a rotary pump to enable rotating the liquid in the channel even during the period that all the inlet and outlet valves are closed. The Fig.1 shows a number of wells (micro-vessels) in the right channel wall in cubic shape with different geometries in length and width (e.g. for the experiment of the next section, channel wall has 10 different wells with diameter of W*L: 10*10um2, 15*25um2, 20*20um2, 10*25um2, 30*25um2, 30*30um2, 35*35um2, 20*40um2, 30*35um2, 10*40um2 where W is the width of cross section and L is the length or depth of the cubic well). These vessels play the role of picoliter chambers which can incubate bio-species-immobilized iron beads (e.g. in the experimental set-up of the next section, biotinylated 2.8 um M270 DYNAL iron beads with 95-mer single-strand-DNA includes 35-mer hairpin, immobilized on the surface of these beads were used, where ROMO is a 35-mer hairpin for DNA strand).

The fabrication process used for this PDMS system is named soft lithography technique [14] and has been done in. The details on the fabrications processes can be found at [13, 14]. Closing the gates is possible through pressurizing this control line (shown as green line in Fig. 1) either with a gas such as air or a liquid such as deionized water.

Since we were limited in the number of pressure inputs from external pump to pressurize the control lines up to eight in our experimental set up, all ten micrometer-size cubic vessels in the right channel's wall could not get separate control lines for each and we just considered two pressure lines for controlling these vessels; as you can see from the Fig.1 we can control three vessels by two control lines, by encapsulation of the third one between two adjacent separate input pressure lines.

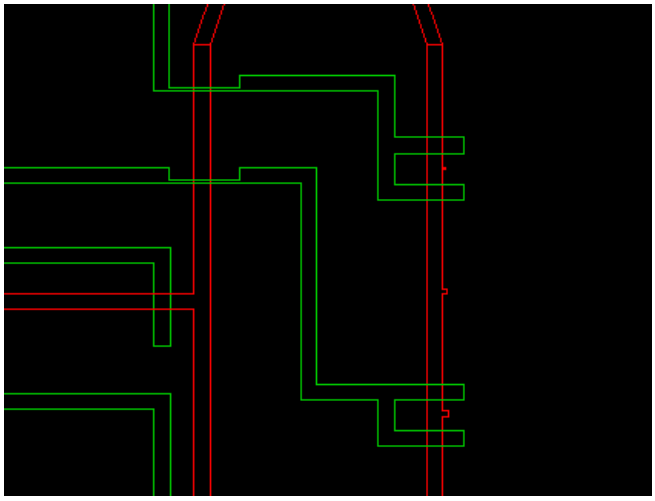

Fig.1. Design of the initial microfluidic system with two layers of control line (green) and flow line (red). The picoliter cells are designed in the right wall.

## SET-UP AND PROPERTIES

Fig. 2 shows the schematic of the system with iron beads in cubic wells in the gate-controlled PDMS micro-channels. As noted in the introduction, there are two special characteristics for this system:

- **A.** The proposed system is similar to the microfluidic system in the 454 platform [7] but instead of fiber optics, the material for the channel is PDMS to be able to gate-control the flow lines by pressurizing control lines [13].
- **B.** Instead of using conventional glass, polycrystalline or Sepharose beads (e.g. Sepharose is a bead-form of agarose (a polysaccharide polymer material extracted from seaweed) which are used widely in the current pyrosequencing industry [7, 18, 19], iron beads are suggested and used in the fabrication process and experimental part of this system.

The effects of the first modification permit these gate-controlled wells for an excellent isolation and effectively reduced cross-talk between adjacent picoliter chambers and preventing one of the main issues of miniaturization in such a microfluidic sensing technology. With the property of iron beads, here mostly two advantages that persuade us for this modification: First, you can have extra control for allocation of the beads in the channel and flow process. [e.g. this is the reason that all the control lines, inlet, outlets and rotary pump in the design of Fig. 1 are drawn in the left side of the wall with vessels in the right side (to test this ability in the experiment), and after that wall in the right side, there is a thin layer of PDMS (after cutting the extra

part) where the external magnet could be placed (e.g. cubic usual magnet or a magnet MEMS actuator) This magnet could be used for positioning of beads inside the channel. In addition, holding the beads with the extra magnet would dramatically affect the washing steps since now you can keep the beads with this external force and then inject liquid inside of the chamber with much higher pressure than before and bring the fluidic base via diffusion and pressurized drift fluidic motion. In addition, by shrinking the size of the beads from a typical diameter of ~30-40 micrometer in conventional pyrosequencing technology to iron beads of a few microns (~1-2.8 um for usual range, we used 2.8 um M270 DYNAL iron beads with original concentration of about hundred millions of bead /ul for our experiments) provides for a better signal to noise ration by providing for enhanced detection sensitivity.

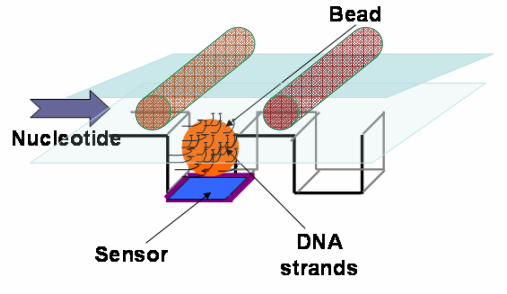


Fig.2. Schematics of the Valve-controlled System with iron beads

## EXPERIMENTS AND RESULTS

In Fig.3, a micrograph of the fabricated microfluidic chip is shown. The chip was placed in a machined attachment of the translation stage of an inverted Olympus IX50 microscope, and the micrograph was taken with the help of an additional 10X lens. The beads, which have been used for the experiments of the initial 100 µm wide and 10 µm high channel platform, are 2.8 um DYNAL iron beads with original concentration of 1E8 beads/µl which have been diluted after injection into the channels The control gates have been manually tested by pressurizing the control valves, with a set-up where pressure can be switched between control lines and between flow lines in the presence of the beads floating in the channel in Stanford Microfluidic Foundry. Having a continuous-flow air supply, a set-up could be built where the pressure supply (in the range of 0-30 psi) to several independent manifolds could be regulated. Each manifold allows us to manually control the supply pressure (ON/OFF) to several independent control lines or flow lines.

In Fig. 4 the micrograph of 2.8 µm iron beads floating in the same channel (e.g. 100µm wide and 10 µm high) is shown. These iron beads can be coated with a layer of biotin and then single-strand-DNAs are attached to this interface layer to bind to the bead surface. The process of immobilization of DNA to surface of beads could be done after PCR multiplication of single-stranded DNA. Effect of the magnet on the motion of the beads inside of the channel during different phases of ON/OFF controlling and incubation of beads in the wells has been further tested.

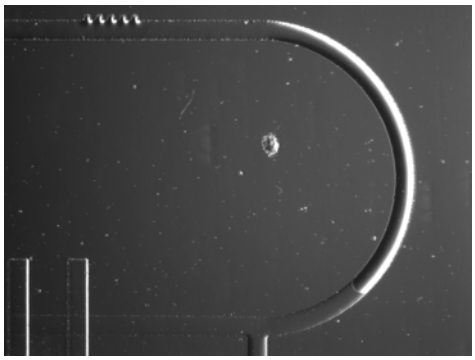

Fig. 3 A Micrograph of the PDMS channel with Gate-Controlled Valves

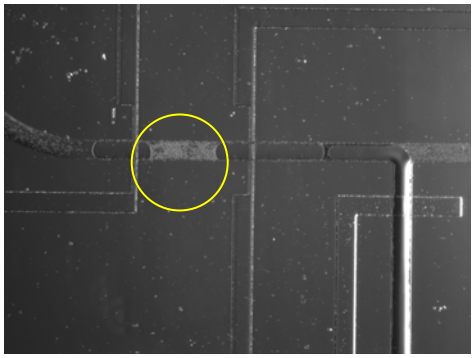

Fig.4. Iron beads in the channel

Fig. 5 shows the phase right after opening control valve for one of the cross sections in the system, when the beads can flow after the pressure is released in the control line. The control line was under pressure of DI-water, and after removing the pressure and releasing the flow line, the floating of iron beads in the channel could be seen in the figure. In addition, leaky valves, which can be used for trapping beads right under the cross section junction of flow and control lines, have also been tested which confirm the theoretical expectation.

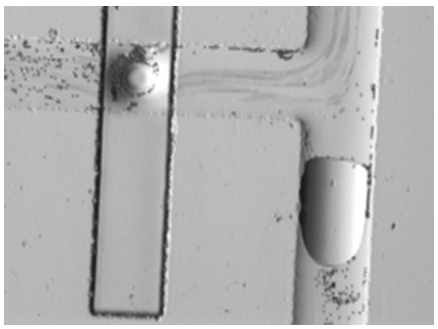

Fig. 5 Flow in the channel: Close valve which traps the beads behind the gate (left), Open valve (right)

## DISCUSSION AND CONCLUSION

In this paper we introduce a novel platform for DNA sequencing-by-synthesis technology,. There are two major changes in the proposed system rather than the conventional pyrosequencing one (e.g. 454 Life Science Corp. technology): First, this platform is based on PDMS gate-controlled microfluidic channels. This leads to encapsulation of the beads in picoliter wells as detection volumes and can significantly improve the signal to noise ratio of the system by preventing cross-talk between adjacent wells. The other modification is the use of iron beads instead of conventional Sepharose or glass beads, gives another degree of freedom on controlling the allocation of the beads by an external magnetic force and makes more efficient washing and injection phases possible, and helps on reducing flow process issues.

This novel DNA sequencing-by-synthesis technology is subsequently used to explore the impact of the design's shape and dimensions of the micro-channels, fluidics parameters, leaky valve trapping mode combined with the role of biotinylated DNA-immobilized iron beads in the PDMS microfluidic system. The resultant potential tradeoffs were discussed which show the advantages of this method in practical applications.

Depends on applications and design schemes, the width and height of the channels have to be modified accordingly for optimizing performance (e.g. for encapsulation of just one bead per well, the width of channel should be rational to the diameter size of the bead while usually the height of the channel is restricted to allow one bead per time from each cross section in the vertical axis). One of the challenges in the ongoing research on valve controllable PDMS microfluidic systems is the diffusion of air bubbles to the channel through the thin membrane between control and flow lines; this case is more likely to happen when the control line is pressurized with air, therefore pressurizing control lines with liquid such as water is preferred to air.

The proposed picoliter micofluidic system could have a very effective role for a number of other bio-species detection and sorting templates such as pathogen detections, antibody-antigen interactions, or real-time PCR detections [6, 15, 16, and 17].

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