Fast DNA Hybridization on a Multi-Sample Multi-probe Microfluidic Microarray Compact Disc

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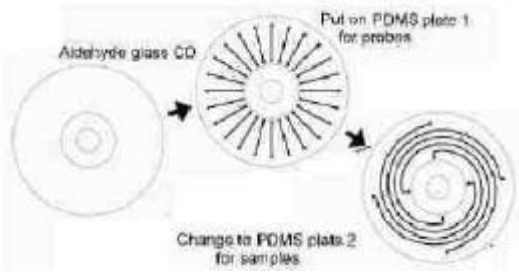

Figure 1: Schematics of the MMA. When the aldehyde glass CD was sealed with plate 1, 24 DNA probes were immobilized. When the CD was sealed with plate 2, 4 samples were hybridized.

ABSTRACT

A microfluidic method has been developed to generate a DNA microarray on a compact disk. Probe immobilization and DNA hybridization are both achieved within microchannels on a microfluidic microarray assembly (MMA). One femtomole of oligonucleotide samples could be detected in 3 min. The reaction times in both processes are much shorter than in conventional methods. It is because the high surface-to-volume ratio achievable in the microchannels facilitates fast surface reaction rates.

Keywords: DNA hybridization, microarray, microfluidic chip, pathogen detection, centrifugal flow

1 INTRODUCTION

DNA microarrays with multiple probes are usually constructed using on-chip synthesis of oligonucleotide probes or by spotting of pre-synthesized probes on the chips [1]. These are expensive methods, and only one sample can be applied on a microarray chip at one time. It is a significant new accomplishment that multiple samples can be tested on a single microarray chip using microfluidic operations.

We have developed a method called the

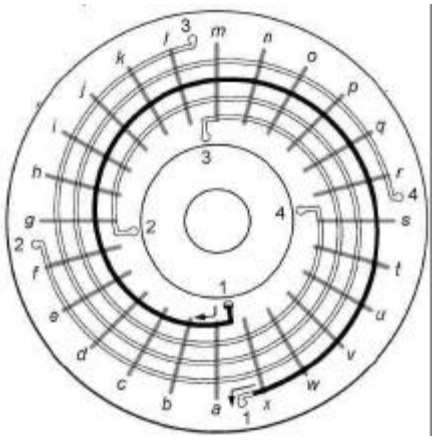

Figure 2: The creation of the DNA microarray. The 24 solid radial lines marked as a-x represent the location of the DNA probe line microarray. The 4 hollow spiral lines marked as 1-4 represent the flow of DNA samples. Liquid flow direction in spiral channel 1 was indicated by black color and the two arrows. DNA hybridizations occur at the intersections between the radial and spiral lines.

microfluidic microarray assembly (MMA). It includes 2 channel plates and 1 common chip (Figure 1). When channel plate 1 is assembled with and sealed against the common chip, liquid flow inside radial microchannels allows the immobilization of a line microarray of DNA probes. When channel plate 2 is sealed against the common chip after the removal of plate 1, liquid flow inside spiral microchannels allows the formation of a spot microarray with DNA samples after hybridization (Figure 2).

Liquid flow was driven by centrifugal force obtained by spinning the CD, as previously described in various reports [2]. In general, centrifugal pumping was used ONCE inside the radial microchannels in plate1/common chip. What is new, centrifugal pumping can be used a SECOND time inside the specially designed spiral microchannels in plate2/common chip. In this work, DNA hybridizations were carried out for 96 samples with 96 probes on the MMA. Hybridizations occur at the intersections of the radial and spiral channels. Small volumes (1μL) and low concentrations (1nM or lower) of multiple samples were used for hybridizations all on one 3.5-inch compact dick (CD).

2 EXPERIMENTAL SECTION

The CD-like glass wafers were chemically modified to possess aldehyde surfaces. PDMS (polydimethylsiloxane) plates were made by molding on silicon wafers [3]. The CD was spun to achieve centrifugal pumping. With a rotation speed of 1800 RPM, the hybridization time was three minutes in room temperature and no washing was needed before fluorescent detection.

The oligonucleotide probes (A and B) have been designed to detect 2 greenhouse fungal pathogen [4]. The probes are first introduced into the radial channels (22μm high, 100μm wide, and 3 cm long). Aminated oligonucleotides (100 μM) in the spotting buffer (0.15 M NaCl, 0.1 $NaHCO_3$, pH 8.5) was used.

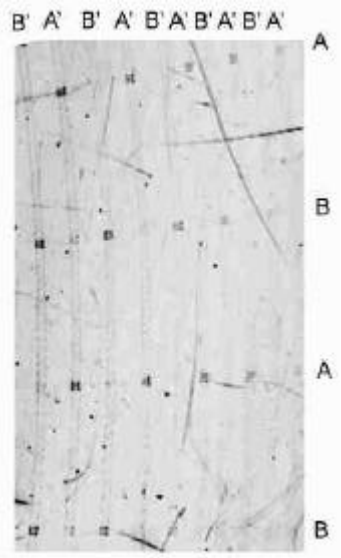

Figure 3: Fluorescent image of the hybridization results at the intersections of the 2 sets of channels. A and B indicate where the 2 animated oligonucleotide probes are immobilized. A' and B' indicate the locations of the respective labelled complementary oligonucleotides.

The sample are complementary oligonucleotides which are Cy5-labeled. The samples were dissolved in the hybridization buffer (1 x SSC, 0.015% SDS).

As the CD was spun, the total volume of the sample (e.g. 1μL) enters the spiral channel (22μm high, 100μm wide, and 20cm long). Therefore, the reactions of the samples with the probes occur in an area of 100μm×100μm. Detection was performed using a confocal fluorescent scanner.

3 RESULTS AND DISCUSSION

Figure 3 depicts a section of the hybridization results of complementary oligonucleotides on the circular microarray. Specific hybridizations (i.e. A to A' and B to B') between complementary oligo-nucleotides were observed.

Full 96-probes-96-samples hybrid-izations were achieved, as shown in Figure 4. The figure was generated after mathematical transformation of the circular microarray data into a common rectangular format. The close-up of a section of the microarray was shown in Figure 5. Here, samples of various concentrations and volumes were used. Since larger amounts of DNA samples were used on the right channels, greater fluorescent intensities were obtained. The right inset represents the results after sensitivity reduction. On the other hand, lower amounts of DNA samples were used on the left channels, and the right inset represents the results after sensitivity enhancement.

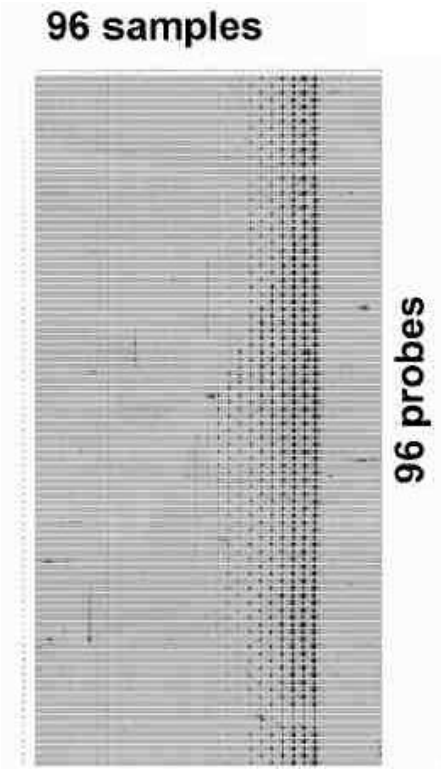

Figure 4: The fluorescent image of a full 96×96 (samples × probes) micoarray after being transformed into a rectangular array.

With a sample flow velocity of ~1-2mm/s, hybridizations were finished in 3 min. The high surface-to-volume ratio of the microfluidic channel provided fast hybridization rates and no high temperature was needed. In addition, the flow continually removed any un-hybridized DNA molecules and no additional washing was nessesary before fluorescent detection.

The concentration detection limit was 1 nM if the sample volume was 1µl (Fig. 5). In this way, one femtomole of oligonucleotide (1 nM) was easily detected. For a low-concentration sample, the same sample could be run in the same spiral channel to achieve a higher signal. Alternatively, a larger sample volume was used. For instance, if 1nM of DNA was detectable with 1µl of sample, detection of 0.1nM will require 10µl (see Fig.5). As the signal increased with the sample volume, detection limit can be reduced to sub-pM concentration.

Although oligonucleotides were used in this work, this MMA method can be easily adpated to other probes, such as cDNA, and samples such as PCR products. Moreover, any surface reactions such as antigen-antibody and protein-protein interactions can be studied.

4 CONCLUSIONS

The impact and significance of the results to the biotechnology fields are to provide a low-cost, user-friendly, multiple-probe and multiple-sample microarray-based method for pathogen detection, clinical diagnostics, discovery of infectious disease genes and oncogenes, pharmacogenomics, to name a few.

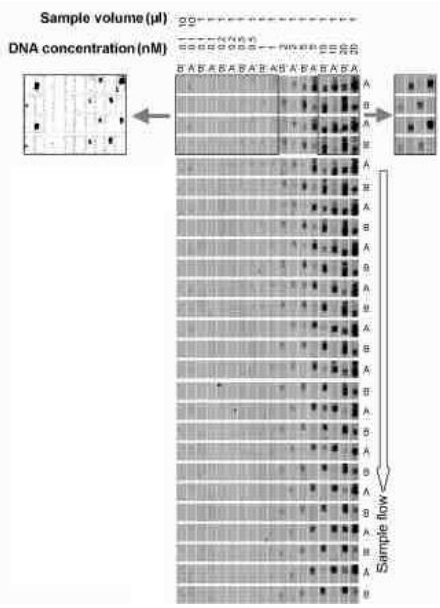

Figure 5: The close-up of a section of the microarray shown in Figure 4. The white hollow arrow represents the sample flow direction in the original spiral channels. Hybridizations were obtained with different DNA sample volumes (1-10 µL) and concentrations (0.1 - 20 nM). The left inset shows a section of the microarray after sensitivity enhancement; the right inset shows a section after sensitivity reduction.

ACKNOWLEDGEMENTS

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REFERENCES

[1] M. Campas and I Katakis, Trends Anal. Chem. 2004, 23, 49-62.

[2] J.V. Zoval and M.J. Madou, Proc. IEEE, 2004, 92, 140-153.

[3] Duffy, D. C. et al. Anal. Chem., 1998, *70*, 4974 -4984

[4] Utkhede R. et al. J Horticulture Sci. Tech. 2002, 77, 62-66 and 604-608.