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Development of a Microarrayer for DNA Chips

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Microarrayer is used to make DNA chip and microarray that contain hundreds to thousands of immobilized DNA probes on surface of a microscope slide. This paper shows the develop-ment results for a printing type of microarrayer. It realizes a typical, low-cost and efficient microarrayer for generating low density microarray. The microarrayer is developed by using a prependicular type robot with three axes. It is composed of a computer-controlled three-axes robot and a pen tip assembly. The key component of the arrayer is the print-head containing the tips to immobilize cDNA, genomic DNA or similar biological material on glass surface. The robot is designed to automatically collect probes from two 96-well plates with up to 12 pens at the same time. To prove the performance of the developed microarrayer, we use the general water types of inks such as black, blue and red. The inks are distributed at proper positions of 96 well plates and the three color inks are immobilized on the slide glass under the operation procedure. As the result of the test, we can see that it has sufficient performance for the production of low integrated DNA chip consisted of 96 spots within 1 cm ^{2} area.

Key words: Microarrayer, Printing Type, Three Axes Robot, DNA chip

Introduction

In particular, with physiochemical stability of DNA, high fidelity and accuracy, and small amount of sample to analyze, DNA typing is bring about revolution in the field of identification. It also plays important roles to understand various characteristics of molecules such as proteins and enzymes within the human body including evolution, structure, and function.

In the conventional gene analysis methods, there are widely two types of Southern blot and Northern blot which make respectively use of interactions between DNA and DNA, and between DNA and RNA (ribonucleic acid) (Purves et al., 1998), but those conventional method can't be applied to study more than dozens of gene at a time. To overcome the those limit, research for the development of DNA detection instruments is actively studied among developed countries such as USA and UK as leading group (Lim et al., 1998; Lanshkari et al., 1997; Kim et al., 1997; Owczarek et al., 1997; Holland et al., 1997). Affymetrix produced commercial DNA chips for the first time. The company founded in 1993 and started with an idea "let's apply the semiconductor technology to development of DNA chips". The first commercial product is used to elucidate the cause of virus's antibiotics resistance against AIDS drugs. Many other companies dreaming INTEL in biochip industry are being founded. Heseq Co. is developing DNA chip technology using filter papers, Synteni co. uses glass materials and Incyte is trying to use ink-jet printer technology for the DNA chip development. But in domestic studies, few laboratories are studying and there are no much results.

This paper shows the development results for a printing type of microarrayer. It realizes a typical, low-cost and efficient microarrayer for generating low density microarray. The microarrayer is developed by using a robot of three-axis perpendicular type. It is composed of a computer-controlled three-axis robot and a pen tip assembly. The key com-

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ponent of the arrayer is the print-head containing pens to immobilize cDNA, genomic DNA or similar biological material on glass surface. The head of tip is made so as to absorb equal amount of probe and the ends of the tips are arranged in parallel with surface of the slide glass for the constant spot size. A simple attenuator is made of stopper and spring to absorb the impact of z-axis transferring to the device at the moment contacting the tips to the glass surface. The wash station and the water drying device are also designed to enhance cleaning of the tips. The dryer is controlled by computer and accomplished rapid air flow around the tips and partial vacuum. The robot is designed to automatically collect probes from two 96-well plates with up to 12 pens at the same time. Wash station and water drying device are also designed to enhance cleaning of the tips. The dryer is controlled by computer and accomplished rapid air flow around the tips and partial vacuum. To prove the performance of the developed microarrayer, we use the general water types of inks such as black, blue and red. The inks are distributed at proper positions of 96 well plates and the three color inks are immobilized on the slide glass under the operation procedure.

The composition of microarrayer

The microarrayer is operated by a procedure as shown in Fig. 1. The DNA or RNA extracted and purified from raw materials is labeled with fluorescent materials or radioisotope. If we observe the result after hybridization with DNA probe integrated in a proper space, then we can obtain an image with brightness according to correspondence of base sequence. We can determine expression of DNA through analysis of the obtained image.

The microarrayer presenting in this article is basically developed by applying three-axis robot technique with print-head in which 12 pens can be loaded in maximum. It absorbs DNA probes, moves to the glass-loaded place, and immobilizes the probes on the glasses. Before spotting the new DNA probes, the cleaning and drying devices remove the remaining ssDNA or impurities by running distilled water and dry aeration. The configuration of the developed microarrayer is shown in Fig. 2.

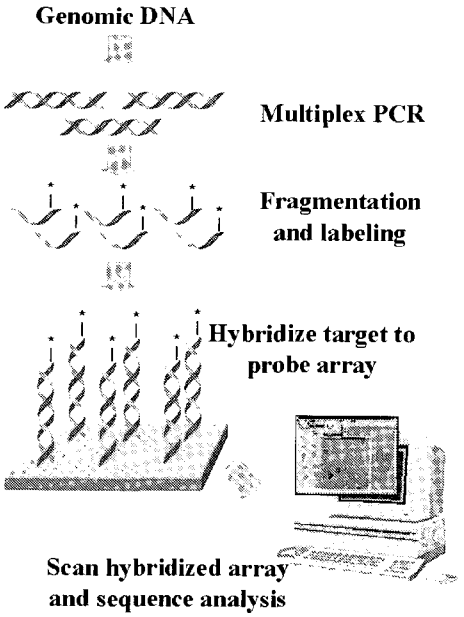

Fig. 1. Procedures of DNA typing using the microarray and/or DNA chip.

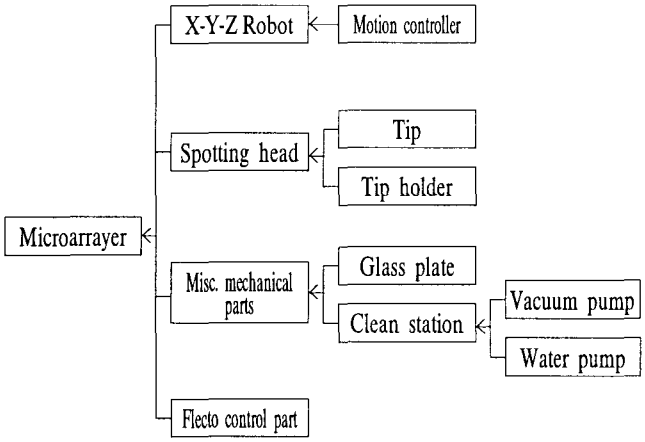

Fig. 2. Configuration of the developed microarrayer.

Microarray/DNA chip spotted on the slide glass

The ideal supporter to immobilize probe can bind probe tightly and facilitate hybridization between testing samples and probes (Cheung et al., 1999). Although membranes such as nitrocellulose and charged nylon are usually used, as the support for the microarrayer the glass is mostly used because of its little inherent fluorescence (Duggan et al., 1999).

The most remarkable difference between conventional cDNA chip and newly developed DNA chip in this study is that oligonucleotides (17~35 bp)

are spotted while phosphate group of ds-DNAs (average 1 kb) combines with poly-L-lysine on the glass in conventional cDNA chip, thus mostly a little part of ds DNA binds on the glass and the rest part can be freely for bound to probe. Because single stranded oligonucleotides have great possibility to bind to poly-L-lysine in whole, there may be no complement sequences to bind to probe. To overcome those problem silylated glass was used instead of poly-L-lysine coated glass. As shown in Fig. 3, this technology makes use of imine bond when aldehyde group of silyated slide glass is easily bound to amine group artificially bound to probe DNA.

Fig. 4 represents a scheme of cDNA microarray using slide glass for microscopy. The cDNA probes spotted on the slide glass by microarrayer are bound to either DNA or RNA of testing object according to the procedure of Fig. 1.

Designs of print-head and pen type tip

The print-head, one of the core techniques of microarrayer, plays a role in immobilizing the probes on slide glass. It should be designed for the constant spot size and absorbing amounts regardless immersing time in probe solution. Fig. 5 shows a schematic representation. Because of its cone-shaped tip, it is very brittle. So, we must use a material keeping the strength of the tip. In addition, to obtain the symmetric spots, the tip should be kept in parallel.

Fig. 6 indicates the principle of spotting. The followings should be taken into consideration in mechanical design. First the impact of z axis moving mechanics should be absorbed at the same time transferring from the state(1) to the state(2). Second, when the state(2) is moved to the state(3), the pressure is discharged. Then since some vibration by z axis movement is transferred, we must charge a proper pressure. In practical usage, when we product the chips with one tip, the productivity is decreased. So, to enhance the effectiveness, the usage of multiple tips is more considerable.

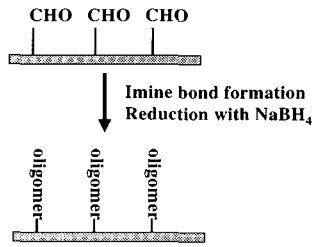

Fig. 3. Principal of bonding the probe on glass surface.

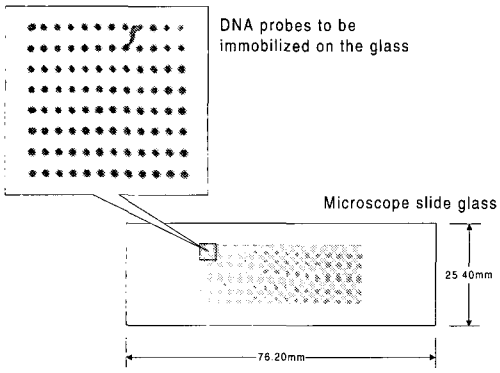

Fig. 4. Schematic representation of the microarray.

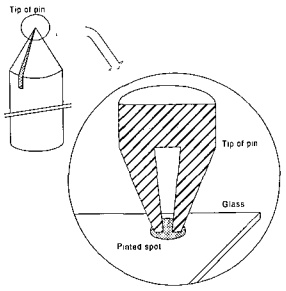

Fig. 5. Schematic representaion of spotting pin and its tip.

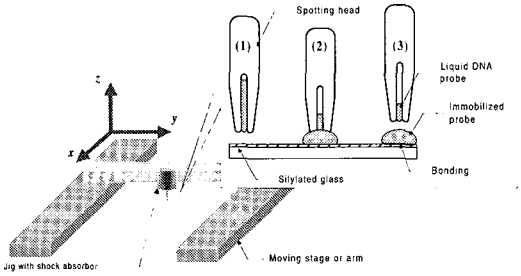

Fig. 6. Spotting mechanism using the pen tip.

The head of tip is made as shown in Photo 1 to absorb equal amount of probe and the ends of the tips are arranged in parallel with surface of the slide glass for the constant spot size. Photo 2 indicates the disassembled print-head. A simple attenuator is made of stopper and spring so as to

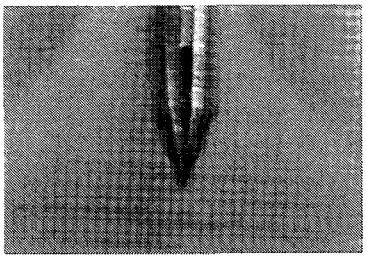

Photo 1. Tip of spotting pen.

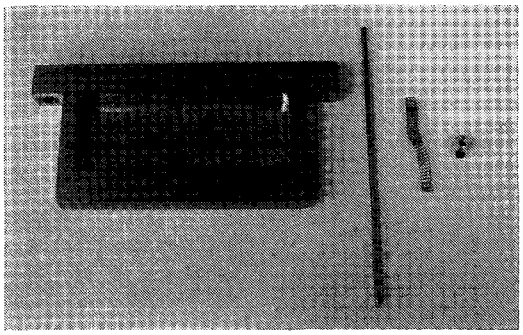

Photo 2. Componets of print-head.

absorb the impact of z-axis transferring to the device at the moment contacting the tips to the glass surface. Furthermore it serves pressurization for decreasing vibration just after spotting operation. The tip holder is composed of a pair of two forms and each holder can be worked by itself. Since each holder can be equipped with 6 tips, 12 spots can be made at the same time.

Three-axes perpendicular robot

The microarrayer is developed by using a robot of three-axes perpendicular type. The capacity and specifications of the robot are shown in Table 1. The external shape of microarrayer is as shown in Photo 3 with fully equipped set up.

Development of various mechanical and electrical equipments

The cleaner to clean the DNA probes remained at the tips and the dryer to dry the tips are set up with one module. The devices are composed of a fluid pump of water and a vacuum pump respectively. Those are controlled by the electrical interface drive module according to the procedure of

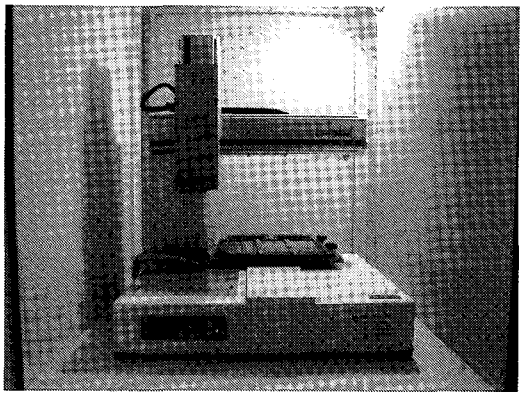

Photo 3. The developed microarrayer applied commericial three-axis robot.

Table 1. Specs of employed three-axis robot

Operating range	X	400 mm
	Y	400 mm
	Z	180 mm
Max speed	X,Y	1,000 mm/s
	Z	500 mm/s
Operating type		PTP, ARCH, Arc interpolation, Position interpolation
Setting up velocity and acceleration		0~100% 0~2 sec
User I/O		Input 16 points Output 24 points
Accuracy of position repetition		± 0.03 mm

Program	Number of input	16 EA×255 line
	Language	40 EA
Spotting weight	Work	5 Kgf
	Head	5 Kgf
Resolution		0.01 mm
Body weight		50 Kgf
Position input type		MDI, Manual, JOG
Number of input position		256
Position detecting type		Incremental encoder
CPU		TMS320C31 DSP
Program input		PC, T/P
Memory back-Up		S-RAM battery

microarrayer operation. The devices for fixing the 96 well plates containing DNA probes and the slide glasses are made by using the reinforced plastic material and stainless steel. The slide glasses and 96 well plates can be conveniently taken on and off by design of simple spring-pressure tool.

Development control algorithm and integrated software

To secure the standard method to store the arrays for both microarray on the slide glasses and the probes of 96 well plates, our own script for GAD (Gene Array Description) is adopted. The software is also developed for spotting on the slide glasses corresponding to the prescribed arrays, for turning on/off of various devices, and for monitoring all the procedure of the operating processes. Photo 5 shows a monitoring screen for an operating state.

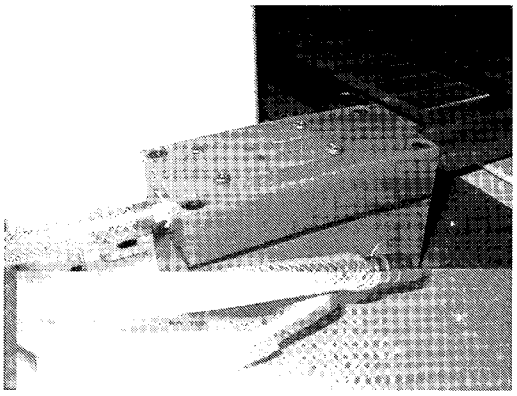

Photo 4. Wash station and dryer.

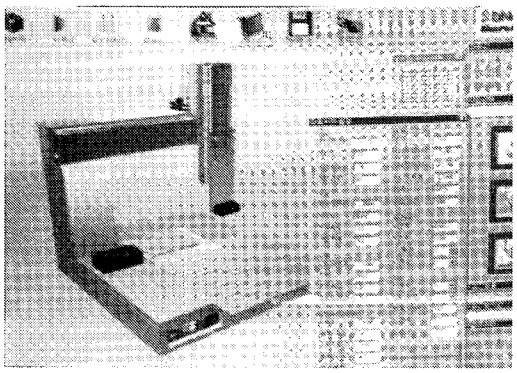

Photo 5. Running screen of microarrayer integrated management software.

Operating test of microarrayer

Interpretation and analysis for the feature of the spots

Since the DNA chip expresses the level of gene expression by the optical strength of each spot, the optical features of the spot should be well understood and the contents can be simplified into a 3D geometrical function as described in Fig. 7. Then, the local grounding level variation due to chip production or image grabber card must be taken into consideration. As the standard parameters to analyze the characteristics of the spots, we choose IOD (Integrated optical density) value with the meaning similar to its mass in an image and MGL (Mean interior gray level) value when threshold level, T is chosen as the following.

$$IOD=\sum_{i=0}^{W}\sum_{j=0}^{H}D(i,j) \tag{1}$$

$$MGL=\frac{IOD(T)}{A(T)} \tag{2}$$

Here, IOD: Integrated optical density,
MGL: Mean interior gray level,
$D(i,j)$: Sample image,
$A(T)$: Area at the threshold T.

Operating test by dyes

To test the performance of the microarrayer developed through the paper, we use a general water type ink which is known well as cheap and visible dyes. The three kinds of inks such as black, blue and red are distributed at proper positions of 96 well plates and the effectiveness of its all operation is evaluated under the control procedure. Photo 6 shows a practical sample result for the spotted

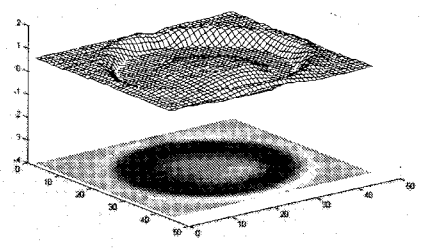

Fig. 7. 3D representaion of one probe spot.

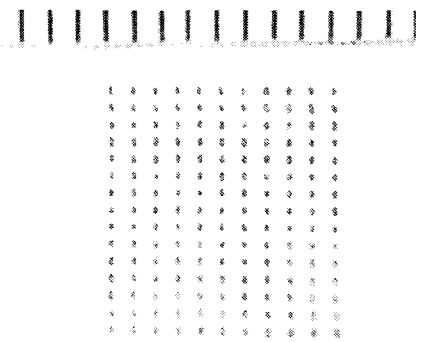

Photo 6. An example of microarray printed by developed arrayer.

microarray, where one scale means 1 mm and the total spot number is 165 (11×15).

Fig. 8 shows the distribution for the spot sizes printed by the developed microarrayer and Table 2 describes the specifications.

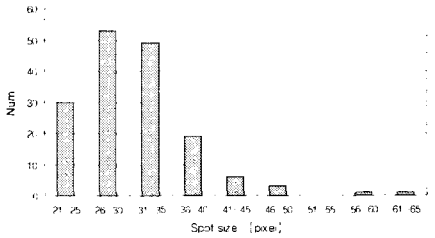

Fig. 8. Distribution of spot size.

Table 2. Spotting specs of the developed microarrayer

Items		Spectrum
Spotting	Size [mm²]	0.34
	Pitch [μm]	290
	Speed [spot/sec]	2.034
	Integration level [EA/cm²]	Maximum 600

Summary

This paper introduced the development result for a microarrayer of print-head type using a perpendicular type of robot. The operating and monitoring software is also developed for spotting on the slide glasses corresponding to the prescribed arrays, for turning on/off of various devices, and for monitoring all the procedure of the operating processes. The cleaner to clean the DNA probes remained at the tips and the dryer to dry the tips were set up with one module. To prove the performance of the microarrayer developed through the paper, we use the general water types of inks such as black, blue and red. Those were distributed at proper positions of 96 well plates and the three color dyes were immobilized on the slide glass. The effectiveness of its all operation was evaluated under the control procedure and also, its effectiveness was proven through the image analysis method. As the result of the test, we can see that it has sufficient performance for the production of low integrated DNA chip consisted of 96 spots within 1 cm² area.

Acknowledgements

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