고정밀 DNA 칩 스캔을 위한 DVD 광픽업의 오토 포커싱 응용

Application of DVD Pick-Up to Precise DNA Microarray Detection

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Key words : English and one line only, Times New Roman 9pt

1. Introduction

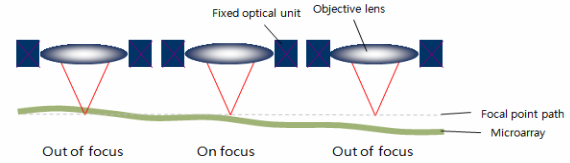
Today, most of DNA or protein microarrays use fluorescent dyes as a DNA marker. In general, the fluorescence-detecting technology has shortcomings, such as weak fluorescent light and a highly controlled positioning mechanism. It is known that the mechanical precision of 1 ㎛ or less and cooling device are required to meet the measurement specifications of commercial confocal scanners. The high cost of the detection mechanism prevents the wide spread in DNA-chip market, especially when high-density diagnostic chips are used. Therefore, the detection technology satisfying both high sensitivity and low cost becomes one of essential factors to expand the DNA-chip market and the related technology and industry [1, 2].

Various researches have used optical storage technology as the detection mechanism for DNA microarrays. It was showed that a CD technology build a detection system with equivalent or even better performances than conventional scanners [3]. And lab on a chip (LOC) system has been developed for application in DNA analysis using a CD disk [4, 5].

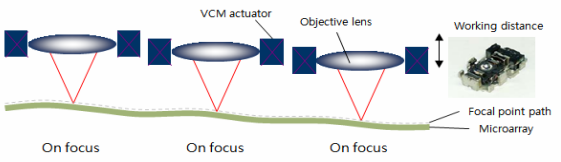
In this paper, a low-cost highly sensitive DNA microarray scanner for fluorescent detection is developed based on the pick-up head of a commercially available optical storage device, DVD. The laser beam of 650 nm, generated by a DVD laser diode, is used for the dynamic auto-focusing as well as the excitation of Cy5 fluorescent dye. The fluorescence intensity emitted from Cy5 dye, is measured by a photomultiplier tube (PMT). In contrast with other microarray scanners, the DVD-based scanner offers the auto-focusing function using the focus error signal (FES) and a voice coil motor (VCM), and it enables fast response, high accuracy and compact size.

2. Auto-Focusing Technology

When scanning a large surface area of a DNA microarray, it is important to keep the distance between the lens and the microarray surface constant with highest accuracy. The main advantage of the DVD pick-up based fluorescent detection is to implement easily a dynamic auto-focusing mechanism by attaching a reflective layer on the commercial microarray. Fig. 1(a) shows that the misaligned or warped microarray can lead to unfocused images when the single point method is used. However, Fig. 1(b) shows the main advantage of the auto-focusing method, which compensates the mechanical tolerance of the DNA-chip surface. The relative position of the objective lens and the microarray surface is determined by 5 degrees of freedom (3 translations and 2 rotations) motions. For the precise detection of the fluorescent marker, the surface translation motions (X and Y) must be less than 1㎛. A DVD optical pick-up, which is widely used in commercial optical storage devices, enables a low-cost sub-micron focusing control [6, 7].



(a) Single point method



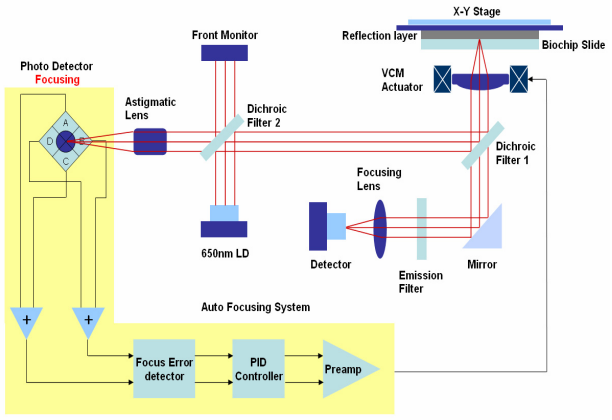
(b) Auto-focusing method

Fig. 1 Comparisons of single point method and auto-focusing method for misaligned or warped microarrays

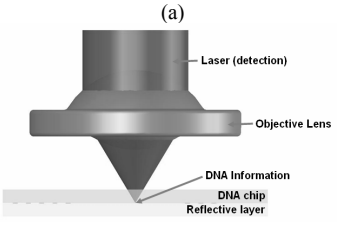
3. Fluorescent Detection Mechanism

Fig. 2 (a) shows the schematic diagram on the optical paths of the proposed system. There are two different optical paths: one is for auto-focusing and the other is for fluorescent detection. A laser diode generates the laser beam of 650 nm wavelength. Most of the laser beam is reflected by a dichroic filter (Chroma Optics Z660RDC), and a part of the laser beam passing through the dichroic filter is used to regulate the laser power by a front monitor sensor. The laser beam transmitting the dichroic filter is focused on the reflective layer, and the reflected beam is finally onto a four-quadrant photo diode (PD) after passing through two dichroic filters. The focus error signal is calculated using the intensities of 4 divided regions of the PD. The PD generates a focus error signal whose magnitude is dependent upon the distribution of the beam spot across its four divided regions. The resulting servo signal is used to drive the VCM actuator in such a way that the objective lens is shifted to a point where its focal point falls upon the spot center. The laser beam focused on the DNA-chip surface excites Cy5 dye with DNA probes, emitting the fluorescent signal of 670 nm. The collimated emission beam is then refocused by detection lens, and passes through a pinhole into the photomultiplier tube (PMT). Analogue electric signal from the PMT represents the intensity of fluorescent signal. The tracking motion is actuated and controlled by a precise positioning stage.

In order to implement the dynamic auto-focusing function, a new microarray slide structure is designed and manufactured by attaching a reflective layer on a commercial DNA microarray slide. Fig. 2 (b) shows the slide structure consisting of two different layers. Since the DNA probe surface is directly attached on the reflective layer, the laser beam is focused on the microarray surface and the reflective layer simultaneously.



(a)



(b)

Fig. 2 (a) Schematic diagram of optical path, (b) DNA-chip structure with a reflective layer

5. Experimental Results

Fig. 3 shows the experimental system to test the fluorescence-detecting performances of the proposed DNA microarray scanner. The focusing motion of the objective lens is automatically

controlled and the focal point falls upon the spot center. The tracking motion is actuated a precise motorized stage to scan the microarray surface. In order to increase the fluorescence signal, the signal to noise ratio (SNR) is to be improved. To determine SNR, the following formula is commonly used:

$$SNR = \frac{\text{Signal intensity} - \text{Background intensity}}{\text{Standard deviation of background}} \quad ...(1)$$

Eq. (1) implies that one may increase the signal or/and reduce the background in order to improve SNR. Typically, the limit of detection (LOD) is defined as the minimum detectable signals for which the SNR is 3. The sensitivity of a DNA-chip scanner is inversely related to the limit of detection – the detection sensitivity increases as the limit of detection decreases.

Fig. 4 shows that the comparisons of SNR for the two cases with and without auto-focusing function. In the experiment, the regular line patterns with constant dye concentration are painted on a slide. Then, SNR was measured along the spot centers with constant fluorescent intensities after adjusting the initial position and light intensity in the spot center. For the auto-focusing case, the magnitude and uniformity of SNR are improved, as shown in Fig. 4. The elevation of SNR is due to the uniform background by the dynamic auto-focusing, the increase of fluorescent signal by reflective layer and the decrease of noise. The weak fluorescent level without auto-focusing is below the limit of detection, and it does not guarantee the minimum detectable signals. Thus, the dynamic auto-focusing mechanism implemented by the DVD pick-up system, is required for the scanner to meet the minimum fluorescence level. The use of the dynamic auto-focusing and reflective layer can remarkably improve the detection performance. On the contrary, the non-uniform profile of SNR without auto-focusing is mainly caused by the background noise, surface variability and misaligned slide surface.

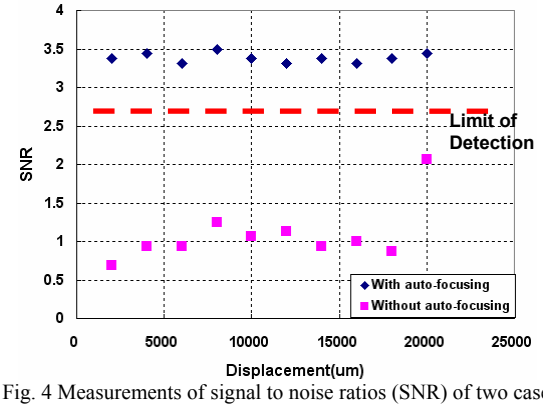
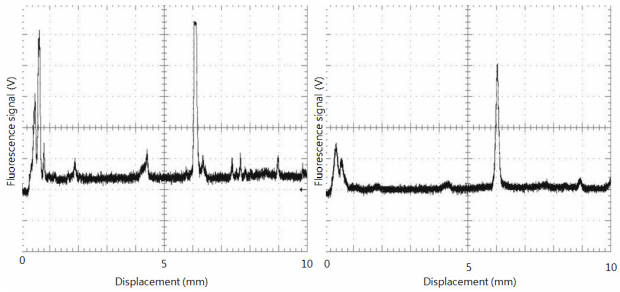


Fig. 4 Measurements of signal to noise ratios (SNR) of two cases: with/without auto-focusing

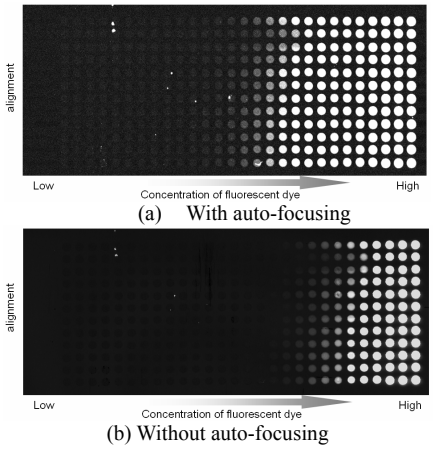
Fig. 5 shows the fluorescent intensities of the auto-focusing and defocused cases. The DNA microarray used in this experiment is a BAC (Bacterial Artificial Chromosome) oligonucleotide chip, which is permitted as a commercial diagnostic DNA-chip from Korea Food and Drug Administration (KFDA). As shown in Fig. 5, the fluorescent intensity and the signal uniformity of the auto-focusing case are much higher than those without auto-focusing. For the case without auto-focusing, there is a remarkable difference between the signal levels measured at two different spot centers, and the non-uniform signal pattern decreases SNR.



(a) With auto-focusing (b) Without auto-focusing

Fig. 5 Experimental results using auto-focusing control

Fig. 6 shows the scanning images of an evaluation microarray slide, which is designed for quantitatively analyzing the dynamic range, detection limit and uniformity of microarray scanners. The experiment uses the scanner calibration slide (DS01) by Full Moon Biosystems Inc. This slide contains array block (32 columns × 12 rows) of dilution series of Cy5 fluorescent dyes The slide can also determine the stability of laser and the alignment of the scanner because each column contains 12 repeats of each dye dilution.. The detection performance of the auto-focusing case is shown to be about 2 times greater than that without auto-focusing in Fig. 6. The non-uniformity caused mainly by the surface variability and misalignment, can be compensated by the dynamic auto-focusing function. This experimental results show that the pick-up based auto-focusing mechanism improves the detection performance compared to conventional diagnostic scanners without auto-focusing.



(a) With auto-focusing

(b) Without auto-focusing

Fig. 6 Detection images of evaluation slide

5. Conclusions

This study has demonstrated a new DNA microarray scanner using a commercially available DVD optical pick-up. The laser beam of 650 nm, generated by a DVD laser diode, is used for the dynamic auto-focusing as well as the excitation of Cy5 dye. The use of the dynamic auto-focusing and the reflective layer attached on DNA-chip slides improves the sensitivity of fluorescent detection. Experiments using a typical evaluation slide have shown that the new DVD-based scanner meet the limit of detection (SNR = 3) which is minimum detectable signal. The new DNA-chip scanner based on the optical pick-up technology, enables high sensitivity as well as low cost.

Acknowledgement

This study was supported by a a grant of Seoul Research and Business Development Program (10816).

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