AIP | Review of Scientific Instruments

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Citation: Review of Scientific Instruments **79**, 035101 (2008); doi: 10.1063/1.2885609
View online: http://dx.doi.org/10.1063/1.2885609
View Table of Contents: http://scitation.aip.org/content/aip/journal/rsi/79/3?ver=pdfcov
Published by the AIP Publishing

Development of a compact optical system for microarray scanning using a DVD pickup head

T. Shimomura,[a),b)] C. Izawa,[a),c)] and T. Matsui[a),d)]
Funai Electric Co., Ltd., 1-1 Minami-watarida-chou, Kawasaki-ku, Kawasaki-shi, Kanagawa 210-0855, Japan

(Received 9 July 2007; accepted 4 February 2008; published online 5 March 2008)

We present a compact optical system using a commercially available DVD pickup head for microarray scanning. Our instrument successfully provides a low-cost, compact, and simple microarray scanning optical system in comparison to conventional ones due to the use of small-sized optical components and the implementation of a simple autofocusing system using an embedded voice coil motor. The performance of this system was validated by using a microarray slide with spots of fluorescent dyes. It was confirmed that our optical head performed satisfactorily and was suitable for practical use in microarray scanners. This result provides evidence of the superiority of our microarray scanning optical system over conventional ones because of its space-saving properties and cost effectiveness. © *2008 American Institute of Physics.* [DOI: 10.1063/1.2885609]

I. INTRODUCTION

Recently, DNA microarrays have been established as a research tool for gene expression studies and applied to various areas such as tumor classifications, identification of genes involved in various diseases, cellular responses, induced responses to external stimuli, and elucidation of biological pathways, and so on.[1–7] DNA microarrays have also attracted significant interest for their applicability to various genetic testing areas such as the rapid diagnosis of infection, drug susceptibility, and even for personal health care in homes.[8–13]

Although the effectiveness of the DNA microarray as a gene analysis tool is now widely accepted, the users are limited to large hospitals or universities. The reason is that the total cost of the system, which comprises the DNA microarray and microarray scanner, is very high at present. In addition to the cost, the optical system of the conventional scanner is often very large and heavy. This cost and complexity hinders the widespread use of DNA microarrays into clinical application. Therefore, the development of a low-cost, compact, and simple microarray scanning system is required for the progress of clinical research studies.

We have developed a low-cost, compact optical system for microarray scanning by using a commercially available DVD pickup head. The optical head is composed of small-sized optical components, including a light-emitting diode (LED) or a laser diode (LD) as the light source, and emission filters that are switched by miniature electromagnetic plungers in front of the photodetector for two fluorescent dyes. The optical pickup lens works as a scanning probe and wobbles in the lateral direction by driving the actuator at an appropriate frequency, and the implementation of a simple focus hold system keeps the microarray slide in focus during scanning. These features make the optical head compact and simple. Thus, our system can provide a space-saving and cost-effective microarray scanning system with good performance.

II. SYSTEM DESCRIPTION

A. Microarray slide

Before describing the structure of the optical head, we briefly introduce the microarray slide that we used for the performance evaluation of our proposed optical system.

For the performance evaluation of our system, we used a $76\times25\times1.2$ mm^3 diamondlike carbon (DLC)-coated glass microscope slide (GENE SLIDE®; Toyo Kohan Co., Ltd., Tokyo, Japan), which has a good track record as a plate for DNA microarrays.

At present, DNA microarrays are often characterized by simultaneous hybridization of two different sets of probes labeled with two different fluorophores.[14] One usually uses Cy™3 and Cy™5 dyes and displays absorption and fluorescence emission spectra, as shown in Fig. 1. In our measurement, corresponding to these fluorophores, graded spotted patterns of Cy™3 and Cy™5 dyes are prepared on the slide as follows: for each fluorescent dye, 7×7 spots are prepared on the slide with six different concentrations of fluorescent dyes, each of which is repeated seven times. The first row is filled with distilled water that acts as a control dye that produces autofluorescence. A schematic representation of these patterns is shown in Fig. 2. The mean value of the spot size is approximately 200 μm in diameter and that of the center-to-center distance is approximately 350 μm.

B. Structure of the optical head for microarray scanning

Figure 3 shows a schematic diagram of our optical head. This unit comprises light sources (LED and LD), an excita-

[a)]Tel.: (+81) 44-329-1221. FAX: (+81) 44-366-6362.
[b)]Electronic mail: shimomura.t@funai-atri.co.jp.
[c)]Electronic mail: izawa.c@funai.co.jp.
[d)]Electronic mail: matsui.t@funai-atri.co.jp.

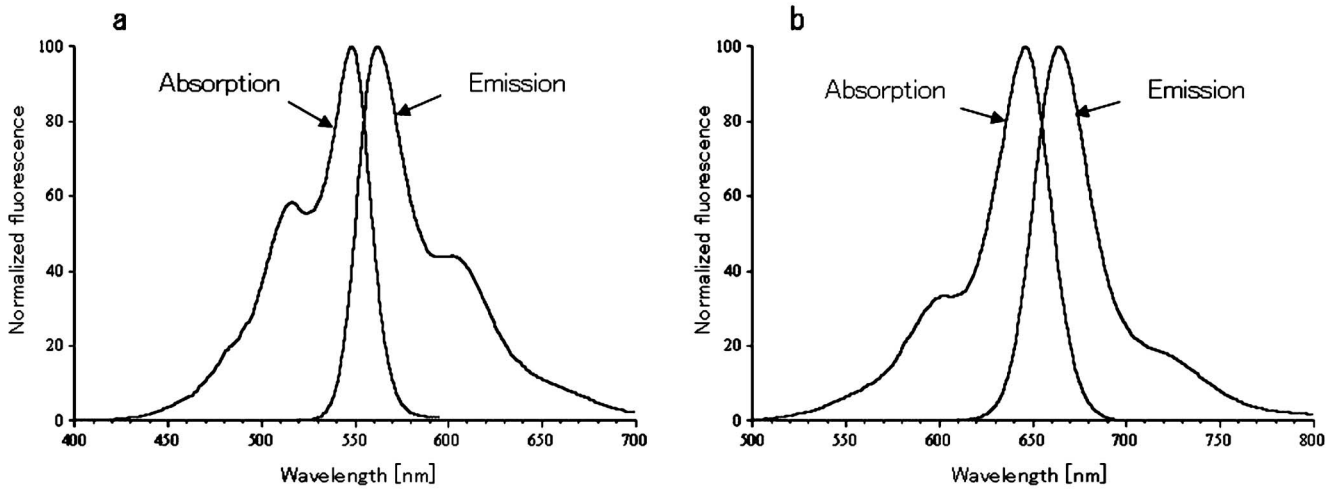

FIG. 1. Normalized absorption and fluorescence emission spectra of Cy™3 and Cy™5 dyes: (a) Cy™3 dye (absorption peak: 550 nm/emission peak: 570 nm). (b) Cy™5 dye (absorption peak: 649 nm/emission peak: 670 nm).

tion filter (EX), collimator lenses (CL1 and CL2), a polarizing beam splitter (PBS), a quarter wavelength plate (QWP), dichromatic mirrors (DM1 and DM2), emission filters (EM1 and EM2), a photodetector (PMT), a sensor lens (SL), a four-quadrant photodiode IC (PDic), an objective lens (OBL), and a voice coil motor (VCM). The unit is designed by using a commercially available DVD pickup head.[15,16] This unit is divided into four main portions: light excitation portion, light-illuminating and condensing portion, wavelength separation portion, and fluorescence detection portion. Next, we will explain each portion in detail.

1. Light excitation portion

Corresponding to the two different fluorophores (Cy™3 and Cy™5 dyes), two excitation light sources with different wavelengths are necessary for the design of microarray scanning optical head.

The light source for Cy™3 is a green LED whose maximum emission wavelength is 525 nm, half bandwidth is 40 nm, viewing angle is 15°, and output power is 5.50 mW (NSPG500S; Nichia Corporation, Tokushima, Japan). The use of LEDs has a significant advantage in terms of size and cost in comparison to conventional systems where the Nd:yttrium aluminum garnet SHG laser is widely used for Cy™3 excitation. The use of LEDs is possible due to the compactness of our optical system.

On the other hand, the light source for Cy™5 is a red LD whose wavelength is 655 nm, beam divergence is 30° in the perpendicular and 8.5° in the parallel direction, and output power is 4.57 mW (SLD1134VL; Sony Corporation, Tokyo, Japan). In order to avoid a wavelength shift of several nanometers due to temperature effect, an autopower compensation circuit is integrated and as a result, the power stability is guaranteed to be 0.4%.

The LED light is collimated (CL1); it passes through an excitation filter (EX) and reflects on the dichromatic mirrors (DM1 and DM2), and it is transferred to the pickup OBL and focused to a small spot on the microarray. The LD beam passes through the beam splitter (PBS) and is collimated (CL2); it then passes through QWP and dichromatic mirror

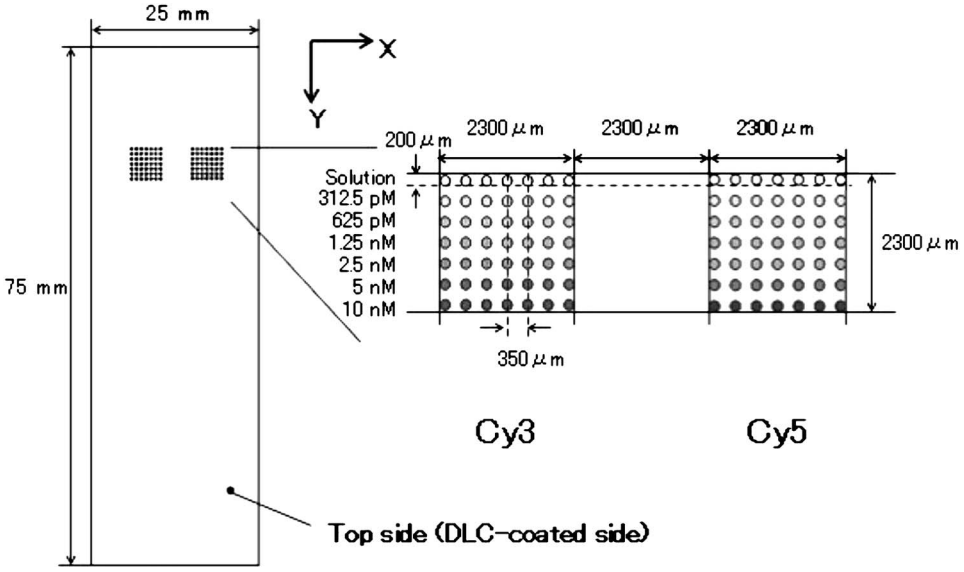

FIG. 2. A schematic representation of graded spotted patterns of Cy™3 and Cy™5 dyes on GENE SLIDE®. Six different concentrations of fluorescent dyes (in columns) are repeated seven times (in rows).

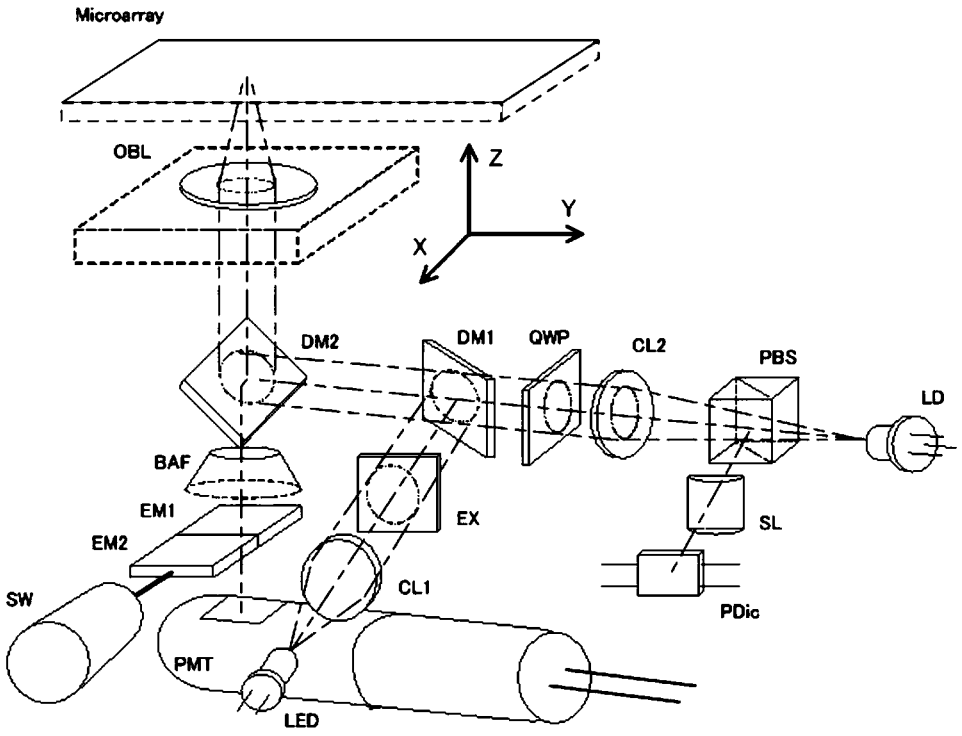

FIG. 3. A schematic diagram of optical head for microarray scanning.

(DM1), reflects on the dichromatic mirror (DM2), and is transferred to the pickup OBL and then to the spot. The measured values of the excitation light powers of the LED and LD after passing through the objective lens are 0.88 and 1.24 mW, respectively.

2. Light-illuminating and condensing portion

In order to obtain high excitation and detection efficiencies for the fluorophores, an objective lens with an appropriate numerical aperture (NA) is required. We used a two-axis moving objective lens actuator (Funai Electric Co., Ltd., Osaka, Japan), as shown in Fig. 4, with NA=0.45/0.60, focal length $f=3.15$ mm, focal depth $h=\pm 0.74$ μm. The objective lens is mounted on the VCM that is used in the driving part of the objective lens along focus and track directions. The ranges of motion are ± 0.70 mm for the focus direction and ± 0.58 mm for the track direction.

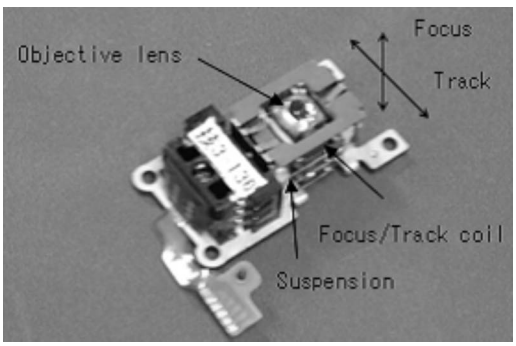

FIG. 4. Two-axis objective lens actuator which uses the voice coil motor (VCM) to drive the objective lens in focus and track directions.

3. Wavelength separation portion

Since the fluorescence signals emitted from the sample are very weak (of the order of picowatts) as compared to the excitation light, we must separate the excitation light from the emission light successfully to avoid the degradation of the detected fluorescence signal integrity. For this purpose, we used DM2, which effectively reflects at the wavelengths of 520–540 nm and 640–660 nm and transmits efficiently at the wavelengths of 560–620 nm and 670–730 nm, so as to transmit only the fluorescence spectrum. In addition, the emission filters (bandpass filters to transmit only the emitted fluorescence wavelength) are placed in front of the fluorescence detecting portion. We used EM1 and EM2 for the wavelength characteristics for Cy™3 and Cy™5 dyes, respectively (Fig. 5) (special order, Nitto Optical Co., Ltd., Tokyo, Japan); they are mechanically switched by miniature electromagnetic plungers with a stroke of 5 mm (P1105A, TDK Corporation, Tokyo, Japan) during the measurement. The maximum chopping frequency of this plunger is 60 Hz. In the output path, a homemade conic-type optical baffle (BAF) with an aperture of 2×4 mm^2 is assembled in order to eliminate the reflections and scattering that generates stray light for the fluorescence detector.

4. Fluorescence detection portion

The transmitted fluorescence signals are detected by a photomultiplier tube (PMT R6356-06; Hamamatsu Photonics K.K., Shizuoka, Japan). This has a good sensitivity in the visible region and the signal-to-noise ratio is excellent. The current generated by the photosensitive surface of the PMT is then amplified approximately 10^5 times inside the multiplier tube and is fed to the amplifier circuit where it is converted into a voltage signal. The output signal is then trans-

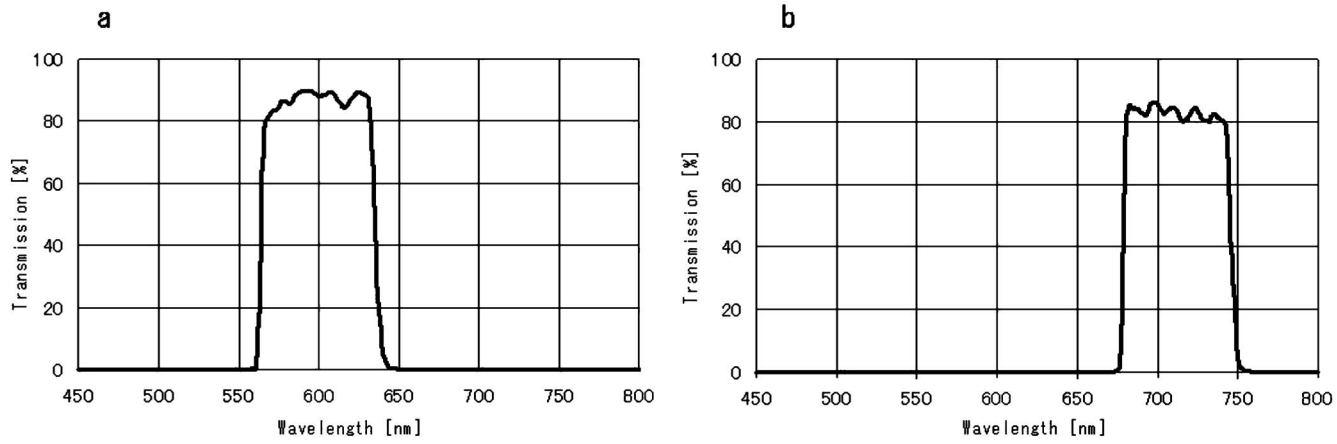

FIG. 5. Spectral curves of emission filters for Cy™3 (left) and Cy™5 (right) dyes. The full widths at half maximum of the filters (a) and (b) are 70 and 65 nm, respectively.

ferred to a NI PCIe-6259 ADC-DAC board (National Instruments Japan Corporation, Tokyo, Japan) with a 16 bit resolution at a sampling rate of 1.25 MS/s and then passed to a recorder. It should be noted that we used a single PMT by adopting the mechanical switching mechanism of emission filters, which has a significant advantage in terms of the size and price of the optical system.

Figure 6 shows an external view of our developed optical head for microarray scanning. The size of the unit is $45(\text{depth}) \times 78(\text{width}) \times 31\ \text{mm}^3$ (height) and its weight is 180 g: it provides "palm-sized portable" microarray scanning optical head.

C. Theory of operation and measurement procedure

Next, we will explain the operation of the objective lens in detail along with the measurement procedure in the experiment.

1. Autofocusing

In our optical system, we can lock the system in focus at all times by use of the moving OBL; the reflected beam of the LD from the tested surface passes through the pickup lens and is reflected by DM2. The QWP shifts the reflected light phase by 90° and then the beam is reflected by the PBS toward the photodiode IC (PDic, M61084FP; Mitsubishi Electric Corporation, Tokyo, Japan). Next, the beam passes through the cylindrical SL that has two focal points, one in front of the four quadrants of the photodiode IC and one behind them, and is detected by the four-quadrant photodiode IC. When these four quadrants are connected so as to produce the combination signal of $[(A+C)\text{-}(B+D)]$, it provides a focus error signal, as shown in Fig. 7, which is subsequently fed back to the VCM for maintaining the focus automatically.[17] This autofocusing motion of VCM makes a significant contribution to the compactness and cost reduction of the microarray scanner because a high-precision and expensive positioning system are not necessary for the focus adjustment.

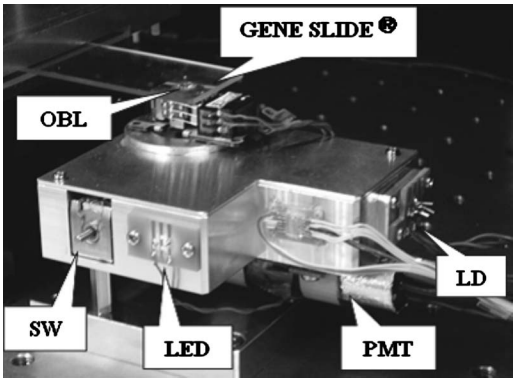

FIG. 6. The external view of our developed optical head. The microarray slide is set to the sample holder which is equipped with an automated *XY*-linear stage.

2. Profile scanning and space addressing

The VCM can be moved along the lateral direction (X direction, as shown in Figs. 2 and 3), for profile scanning by providing a high-precision triangular current signal with an appropriate frequency F_1 to the input ports for tracking. The relationship between the input tracking servo voltage and the displacement output of the OBL is studied for $F_1 = 20$ Hz by

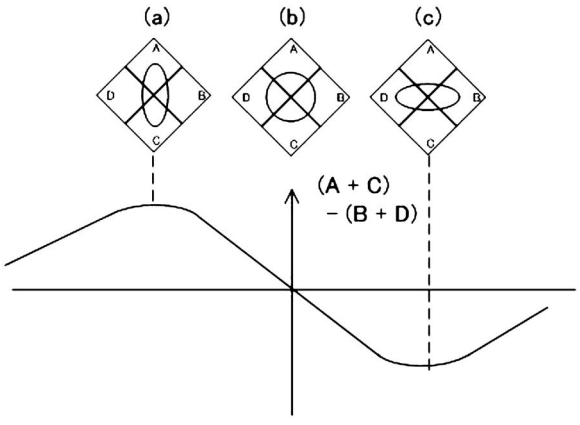

FIG. 7. Illustration of the autofocusing principle. The images of the returned beam on the four-quadrant photodiode IC form an elliptical spot when the surface is out of focus [(a) close, (c) far] and circular when the surface is in focus [(b)]. The combination signal of $[(A+C)\text{-}(B+D)]$ provides an S curve.

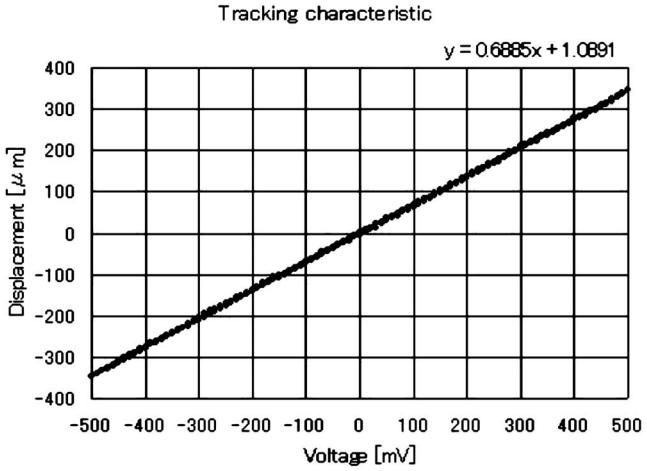

FIG. 8. The diagram of profile scanning characteristic of the VCM. Giving the input ports for tracking about every 10 mV with frequency 20 Hz and recording the displacement of the object lens. It can be seen that good linearity can be obtained in the range up to ± 300 μm.

using laser Doppler vibrometer with a fringe count displacement unit (LDV-1620 and LV-0120; Ono Sokki Technology Inc., Kanagawa, Japan), and the result is shown in Fig. 8. It shows that good linearity can be obtained in the scanning range up to ± 300 μm, the resolution is less than 10 μm, and the accuracy is better than 10 μm.

However, we must prepare a *XY*-linear stage in order to cover the entire spotted area on the slide because the scanning range of the VCM in the tracking direction is too small to scan the entire spotted range in the *X* direction. Thus, in the measurement, we cover the entire spotted area by moving the position of the microarray slide on the holder equipped with a fully automated *XY*-linear stage (SGSP26-100; Sigma Koki Co., Ltd., Tokyo, Japan. The resolution is 4 μm and the accuracy is 10 μm both in the *X* and *Y* directions), we the objective lens mounted on the VCM wobbles in the *X* direction in sync. Accordingly, the microarray image acquired by using our optical head scanner is a composite of several "strip-shaped" scanned images.

In practice, we use high-precision triangular signal sweeping from 436 to −436 mV with a frequency of 20 Hz for the input ports for tracking; this signal is generated from a NI PCI-7344 servo/step motion controller board (National Instruments Japan Corporation, Tokyo, Japan) with a 16 bit resolution at a sampling rate of 4 MHz. This motion in the *X* direction is synchronized with that in the *Y* direction of the automated *XY*-linear stage. The signals acquired from the photodetector (PMT) are combined with the positional information of this *XY*-linear stage and are then treated by using real-time data acquisition software based on the LABVIEW™ programming tool (National Instruments Japan Corporation, Tokyo, Japan). Therefore, by gluing several strip-shaped images, we can scan the microarray slide with a resolution of 10 μm both in *X* and *Y* directions while keeping the system in focus.

III. EXPERIMENTAL RESULT

In this section, we show the result of the performance evaluation of our developed optical head for microarray scanning; the evaluation is performed by using the graded spotted patterns of Cy™3 and Cy™5 dyes prepared on GENE SLIDE®, as described in Sec. II A.

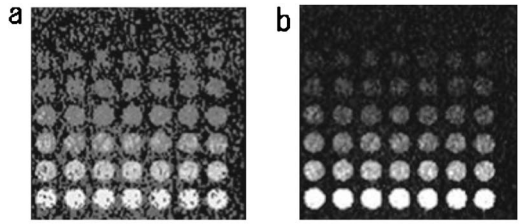

FIG. 9. The scanned images of spotted pattern on GENE SLIDE® for Cy™3 dye(left) and Cy™5 dye(right), respectively.

Figure 9 shows the scanned images of the spotted pattern on GENE SLIDE® that are measured by using the developed optical head. The left image is for the spotted pattern of Cy™3 dye and the right one is for that of Cy™5 dye. The images are produced in a 16 bit tagged image file format.

The measurement time for scanning a spotted area (2300 μm $\times$ 2300 μm $\times$ 2) with a 10 μm resolution was approximately 100 s. In the measurement, the movement between columns was sequentially generated by the motion of the linear stage in the *Y* direction according to the instructions of the program. It should be noted that we kept the LD and focus servo on during the measurement for both dyes in order to maintain focus and we had to turn the LED on and off in synchronization with the switch of the emission filters depending on the dye. This simple mechanism has a significant advantage for high-speed scanning in practical application because in most practical cases, two samples labeled with Cy™3 and Cy™5 dyes are allowed simultaneously to hybridize with the corresponding probes on the slide before the relative abundance of the two fluorescent dyes for each spot is measured.

We examine the fluorescence intensity per spot that is obtained by averaging over the seven "horizontal" spots for each of the rows and establish the system response to different concentrations of Cy™3 dye and Cy™5 dyes. The results are shown in Fig. 10 along with those for typical commercial scanner (DNAscope™ V; Biomedical Photometrics Inc., Ontario, Canada).[18] The response curves of our optical head are linear over the measurement range of 312.5-10 nM and are sufficient to detect a concentration of 312.5 pM for both Cy™3 dye and Cy™5 dye. The quantitative limits, defined to correspond to the brightness signal approximately 10σ above the background level represented by the blank solution, are approximately 500 pM for Cy™3 dye and 800 pM for Cy™5 dye. Thus, our optical head has an advantage over the commercial scanner with regard to the sensitivity in the low-concentration region for fluorescent dyes. This result shows that our developed optical head using an optical pickup lens shows satisfactory performance for practical application for microarray scanner.

In our system, data are acquired with a pixel size of 10 μm and a fluorescent spot with a diameter of 200 μm is

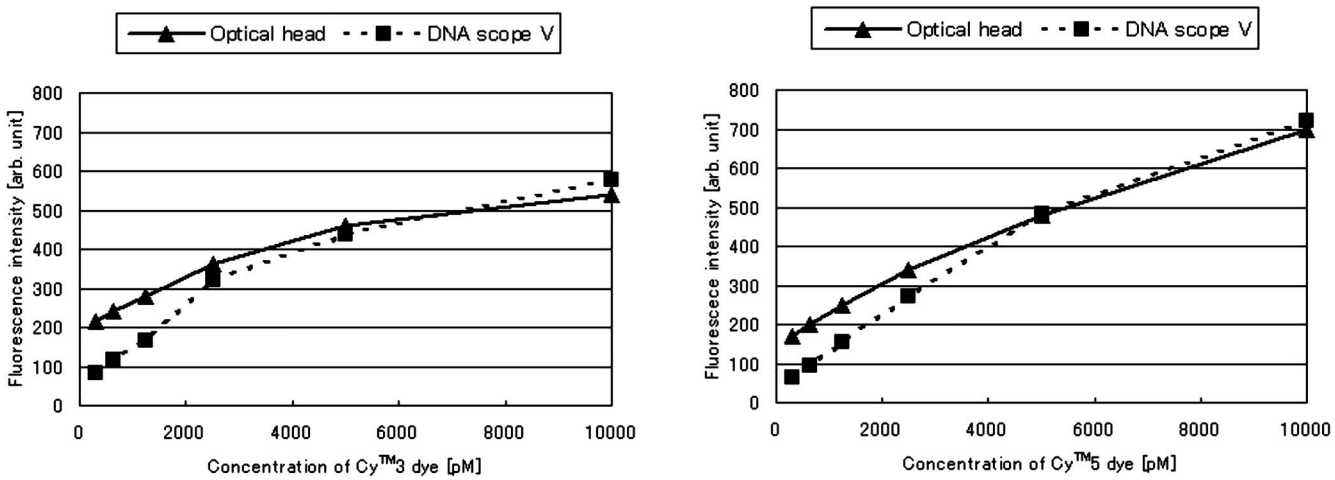

FIG. 10. System response to different concentrations of two fluorescent dyes, i.e., Cy™3 dye (left) and Cy™5 dye (right). The solid lines denote the results of our developed optical head and the dotted lines denote those of typical commercial scanner.

imaged onto ~315 pixels. We can measure up to approximately 1500 spots/cm^2, each with a mean diameter of 200 μm and a center-to-center distance of 250 μm. This is restricted by the resolution of the *XY*-linear stage. Since the spot size of the excitation light is less than 0.87 μm, the detection of spots with a higher density would be possible by using a high-resolution linear stage.

IV. SUMMARY AND DISCUSSIONS

We have developed a low-cost, compact, and simple optical system for microarray scanning by using a commercially available DVD optical pickup head. The performance of this optical system was evaluated by using a DLC-coated glass microscope slide, and the result was compared with that of a typical commercial microarray scanner. It was then confirmed that our optical head showed satisfactory performance for practical use in microarray scanners. This result shows that commercially available DVD optical pickup head can be successfully applied to microarray scanners. Since optical pickup head can provide inexpensive and palm-sized portable microarray scanning optical heads, we can expect it to make a significant contribution in increasing the use of genetic diagnosis and in the development of personalized medicine by the use of microarrays in future.

Finally, we comment on the spotted format of the microarray. It is convenient for our small optical system to consider a strip-shaped spotting format so that all the spots fit into a scanning area of 1×9 mm^2; further, a "spiral-shaped" spotting format on the CD/DVD disk-shaped array is also convenient because we can cover all the spots by moving the mechanical stage only in one direction at a low speed while the objective lens wobbles in the track direction.

ACKNOWLEDGMENTS

The authors would like to thank Professor Furihata and Toyo Kohan Co., Ltd. for providing us a GENE SLIDE® with fluorescence dyes and useful comments.

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