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Development of the Compact UV Illuminator with Single UV Lamp

(Pembangunan Iluminator UV Padat dengan Satu Lampu UV)

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ABSTRACT

This paper presents a low-cost method of constructing the compact UV illuminator, which is considered as an important component of a gel documentation system. The procedure involves using a smallest-possible UV lamp and a motor which moves the UV lamp in the UV illuminator instead of conventional 4 UV lamps. A comparative analysis of images produced by using the commercial gel documentation system and our prototype was carried out. These comparisons were done in real DNA gel as well as a reference plate made of quantum dot. The plate was composed of the chambers filled with various densities of the quantum dot instead of the Agarose gel containing the ETBR in order to increase the accuracy of comparison and the convenience of experiments. Despite the use of only 1 UV lamp, the proposed system demonstrated a similar imaging performance compared with the conventional gel documentation system equipped with 4 UV lamps, resulting in the great reduction of the system cost.

Keywords: DNA detection; gel documentation system; gel image analysis; quantum dot; UV illuminator

ABSTRAK

Kertas ini membentangkan kaedah kos rendah membina iluminator UV yang padat dan dianggap sebagai satu komponen yang penting dalam sistem dokumentasi gel. Prosedur ini melibatkan penggunaan lampu UV terkecil dan motor yang menggerakkan lampu UV dalam iluminator UV dan bukannya lampu konvensional 4 UV. Satu analisis perbandingan imej yang dihasilkan dengan menggunakan sistem dokumentasi perdagangan gel dan prototip kami telah dijalankan. Perbandingan ini telah dijalankan dalam gel DNA sebenar serta plat rujukan buatan daripada dot kuantum. Plat tersebut terdiri daripada ruang dalam yang dipenuhi dengan pelbagai kepadatan dot kuantum dan bukan gel Agarosa yang mengandungi ETBR untuk meningkatkan ketepatan perbandingan dan kemudahan uji kaji. Walaupun hanya menggunakan 1 lampu UV, sistem yang dicadangkan menunjukkan prestasi imej yang sama berbanding dengan sistem dokumentasi gel konvensional dilengkapi dengan 4 lampu UV, seterusnya mengurangkan sistem kos.

Kata kunci: Dot kuantum; gel analisis imej; pengesanan DNA; sistem dokumentasi gel; UV iluminator

INTRODUCTION

In general, DNA detection involves the following 4-step process: DNA extraction, DNA amplification, electrophoresis and gel image analysis (Kodziusa et al. 2012; Salm et al. 2011; Wu et al. 2012). Each step requires expensive equipment (Bajla et al. 2005; Machado et al. 1997; Ye et al. 1999). Gel documentation system (simply called Gel Doc) is very expensive equipment, mainly because of its camera and UV lamps. Thus, it is necessary to conduct the experiments to identify alternative setups that would reduce the cost for gel documentation by using the same principle of UV illumination. The investigations generally involve performing gel image analysis, considering various types of UV lamps and cameras as well as the prices of each component. However, only a few studies on this nature have been conducted (Goldmann et al. 2001; Porch & Erpelding 2006). We used cheap camera instead of the expensive DSLR camera which is used in the conventional Gel Doc system in our previous work (Lee et al. 2013). The conventional Gel Doc system has several UV lamps in the UV illuminator to make the gel image uniform and retain the sufficient light intensity. However, if we can reduce the number of the UV lamps, we will be able to make Gel Doc cheaper and more compact (Lee et al. 2014).

We proposed the mechanism where the UV lamp was moved by motor in uniform speed. This study showed that the non-uniformity can be overcome by moving the UV lamp with motor in uniform. Qualitative and quantitative comparison of the image quality with the existing UV illuminator equipped with 4 UV lamps was performed.

Generally, the performance comparison of this type was done by capturing the image of the DNA gel which was processed in extraction, amplification and electrophoresis. However, this process cannot be assured to produce the same result in respect of the brightness of the image and also does not guarantee the time repeatability. Furthermore, the whole DNA gel processes were required whenever the comparison was performed. In order to overcome these problems, we made the absolute reference plate using the chamber filled with quantum dot instead of the real

DNA sample. This reference plate helps getting the image without a biochemical procedure and was more time-invariant and the experiment was more convenient. The comparison analyses were performed using both real DNA samples and the quantum dot plate.

Materials and Methods

The Gel Doc tested in this study had a DSLR camera (Canon EOS 450D) and consists of a camera box, dark box and a UV illuminator as shown in Figure 1. Given that Gel Doc is a device that captures the UV-induced illumination of fluorescent material within the gel, the dark box was constructed to restrict light from outside and to ensure proper photo documentation of fluorescent DNA bands. In order to make the UV illuminator compact, the smallest-possible UV lamp was used; a typical UV illuminator consists of 4 UV lamps and has 4 ballasts as shown in Figures 2 and 3. The UV illuminator used in our experiment could be used in the existing Gel Doc as well as in the miniature version proposed in this paper. Furthermore, for the sake of user convenience, four sub UV lamps switch was added to the device as shown in the right bottom corner of Figure 2. Figures 2 and 3 are schematic diagrams of the top-view and side-view block diagrams of conventional UV illuminator, respectively, which showed where the lamps and ballasts were positioned.

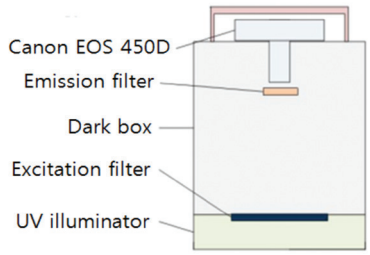

FIGURE 1. The smallest Gel Doc block diagram

In order to make the small and cheap UV illuminator, we adopted a single UV lamp instead of 4 lamps in the conventional system. We solved the UV light uniformity problem which can be caused by single UV lamp through moving the lamp with motor. Figures 4 and 5 show the block diagrams of UV illuminator equipped with motor.

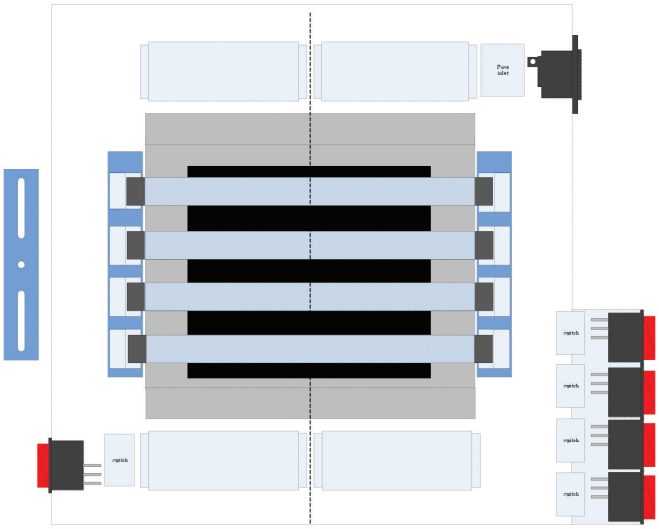

FIGURE 2. Conventional UV illuminator top-view block diagram

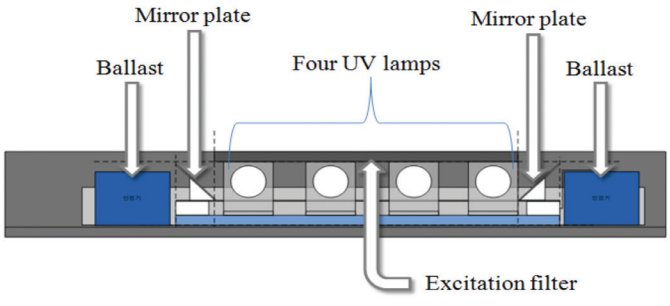

FIGURE 3. Conventional UV illuminator side-view block diagram

This equipment includes a motor, UV lamp, timing belt, pulley and sliding guides. The pulley and the motor were installed by drilling two holes on the acrylic plate and the lamp socket fixture base had slippery feet which were guided by sliding guides for smooth moving of the UV lamp. The stepper motor was adopted to control the moving of the UV lamp and Table 1 shows its specification. Figure 6 shows the circuit diagram for driving the motor.

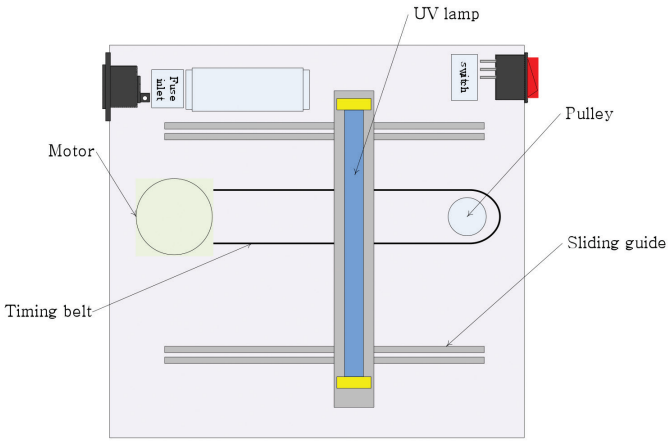

FIGURE 4. Proposed UV illuminator top-view block diagram

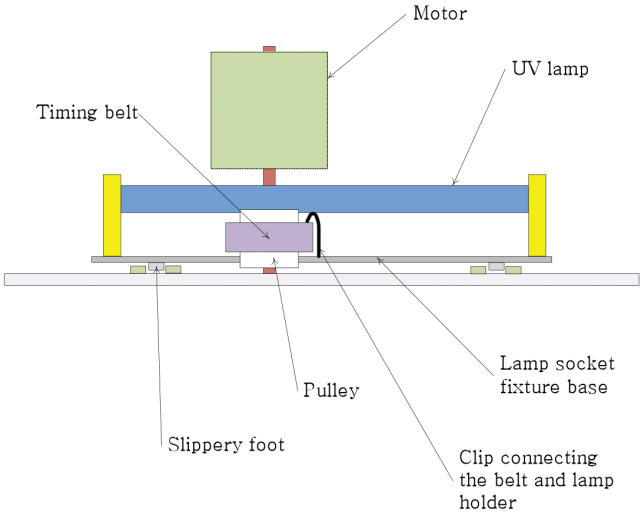

FIGURE 5. Proposed UV illuminator side-view block diagram

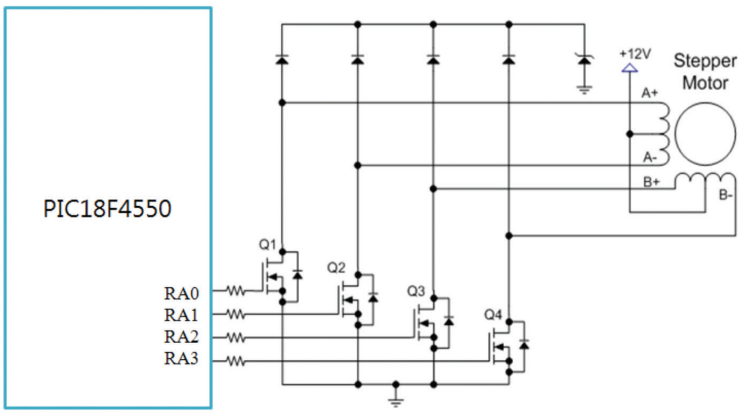

FIGURE 6. Circuit diagram for driving the motor

TABLE 1. Specification of the step motor

Product name	A4K-M245
Manufacturer	Autonics
Step angle	1.8° / 0.9°
Method	Unipolar

TABLE 2. Full-step pattern

A	B	Q1	Q2	Q3	Q4	
-	-	0	0	1	1	1.8°
-	+	0	1	1	0	1.8°
+	+	1	1	0	0	1.8°
+	-	1	0	0	1	1.8°

According to the motor's specification, Unipolar Pattern is divided into Half-Step pattern that is rotated by 0.9° and Full-Step pattern that rotates 1.8° in each step. We used the Full-Step Pattern in this paper shown in Table 2. To control the step motor, we used four ports of PIC18F4550 and equipped it with MOSFET, diode and Zener diode. The proposed UV illuminator has 1 UV lamp and 1 ballast, replacing 3 UV lamps and 3 ballasts by the motor related components such as a motor, a pulley and two sliding guides. The cost of the added components was comparable to that of one UV lamp. Figure 7 shows the screen shot of the windows program with which the stepper motor was controlled. The GUI (Graphic User Interface) shows the motor control functions such as direction, delay and revolution control. As EOS utility capture program was used to take a photo of the gel or the reference plate, the above stepper motor control program sent the windows button event to the EOS utility program to synchronize the camera shooting with the motor start.

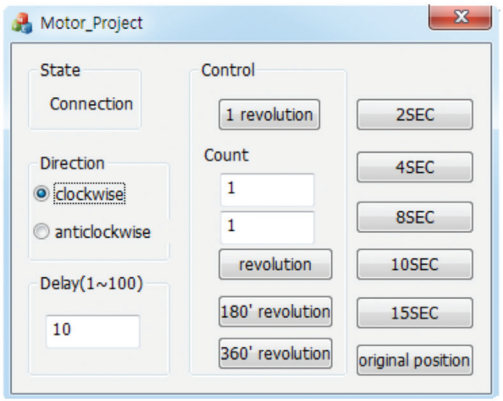

FIGURE 7. Stepper motor control program

The conventional UV illuminator with 4 UV lamps used the exposure time of 0.5 s. However we need 2 s of the exposure time for our UV illuminator since the proposed system has just 1 light source while the conventional system has 4 UV lamps, that is, four times darker than the conventional system. The other settings of the camera were the aperture of 5.6 and ISO of 1600.

The reference fluorescent plate was implemented to emulate the DNA gel. The reference plate has the colloidal quantum dots instead of the ordinary fluorescent dye for time-invariance. Figure 8 shows the block diagram of the chambers of the reference plate. The chamber was composed of acrylic base, double-sided tape in which the chamber shape was cut out and the polypropylene film which covered the chamber and had two holes for the inlet and outlet. The colloidal quantum dot (YVS1001, 300 nm excitation and 607 nm emission, Sun Innovations, Inc.) was diluted to various concentrations and injected into the corresponding chambers and the inlet and outlet holes were sealed with a tape. The dilution factors were 1/2, 1/4, 1/8 and 1/16.

The subjects such as the DNA gels and the reference plate were photograph twice, one with the conventional gel documentation system with 4 UV lamps and the other with the proposed UV illuminator with a single UV lamp. To accomplish this, an existing gel documentation system divided into two parts: the UV illuminator and the upper module consisting of the dark box and the camera box. The subjects were fixed on the UV filter of the dark box to prevent from being misplaced against the camera while moving from the UV illuminator of the existing gel documentation system to the proposed one. The upper module was very carefully moved because the quantitative image comparison was based on the pixel-wise image subtraction.

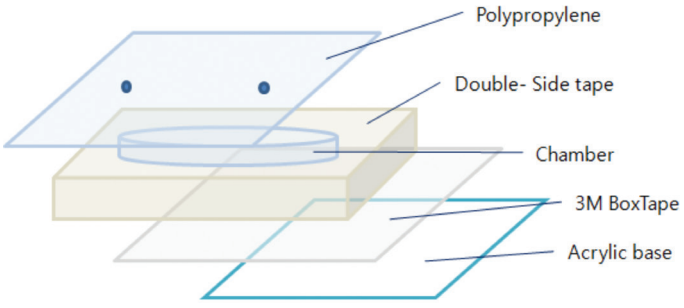

FIGURE 8. Block diagram of a chamber in the reference plate

Results and Discussion

Two kinds of experiments were performed for verifying the proposed UV illuminator: one with the real DNA sample and the other with the reference plate made of colloidal quantum dots. In the real DNA experiment, the images captured were cut to the appropriate size and then converted to the gray scale using MATLAB. The gray-scale transformation and the image registration were undertaken to generate more precise image information that was subsequently used for comparative analysis. Figure 9 shows the processed image of using the conventional Gel Doc. Figure 10 was obtained by using the proposed UV illuminator of using the motor.

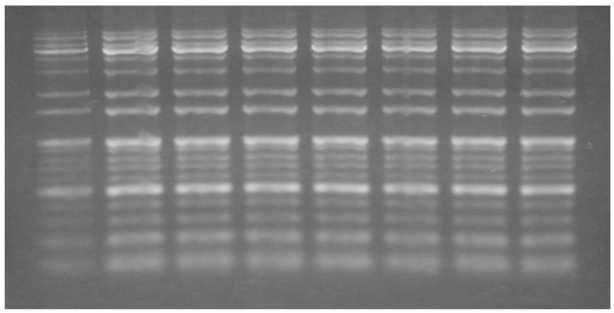

FIGURE 9. Gel image captured by the conventional 4 lamp UV illuminator

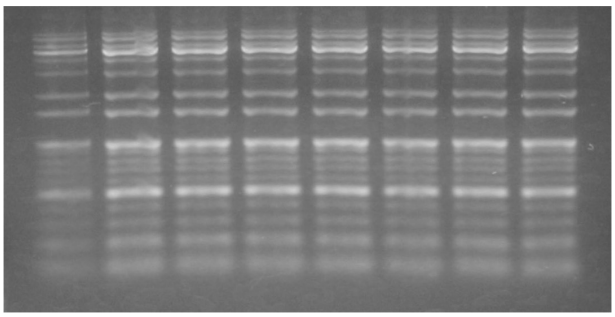

FIGURE 10. Gel image captured by the proposed UV illuminator

In order to ensure an accurate comparison, we converted the images to gray-scale. Furthermore, we used the stretching method to correct the pixel values of a specific area. To compare the two images obtained from the proposed system and the conventional system, we applied the subtracting operation between two images on the pixel by pixel basis. Two trial were performed for the comparison. The upper image and the lower images in Figure 11 are the contrast enhances versions of the difference images from the images captured in the first and the second try, respectively. For the quantitative comparison, the means and the standard deviations of the different images were shown in Table 3. Table 3 shows that the standard deviation of the intensity difference does not exceed 0.3 of 256 levels of intensity. The visual inspection of the difference images in Figure 11 and the statistics of the images showed that the images from the proposed UV illuminator were comparable to them from the conventional one.

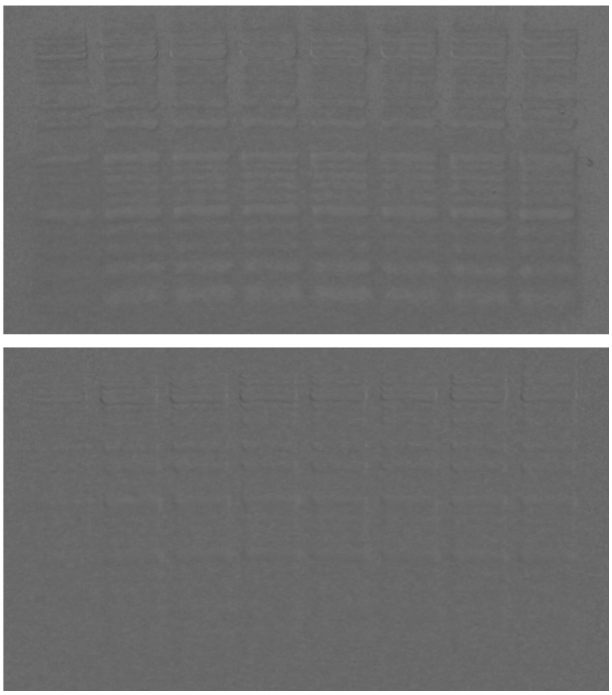

FIGURE 11. The differences of gel images captured from the conventional UV illuminator and the proposed one (top: the difference image for the first try, bottom: the second try, contrasts were enhanced for the visibility)

TABLE 3. Comparative analysis of the captured image

Tries	Mean	Standard deviation
1st trial	0	0.3
2nd trial	0	0.2

Figures 12 and 13 show the images of the reference plate captured by the conventional and proposed UV illuminator, respectively. In the figures, from the left chamber, the original solution, 1/2, 1/4, 1/8 and 1/16 dilutions of the colloidal quantum dots were inserted as indicated in the bottom of the individual chamber image. The median intensities of the centers of the chamber images of the figures were listed on Table 4. The figure shows the similar image qualities and the median intensities of the chamber images were also similar to each other showing the correlation of variation (CV) was less than 13.6%. These also verified that the proposed UV illuminator performs comparable to the conventional one.

To show how the intensities of the images of the reference plate emulate the real DNA gel, a DNA gel image was captured and shown in Figure 13 with the

conventional 4 lamp UV illuminator. Table 5 shows the median intensities of the band images in Figure 13. The mean intensity of the band images was 95.5 and it was between the intensities of the 2nd and the 3rd chamber images from the left. The images in Figures 12 and 13 show the similar visual quality to that in Figure 14 and the median intensities of DNA bands and the chamber images of the reference plate also show the analogical tendencies. These promised that the reference plate well emulated the ordinary DNA gels. The reference plate was imaged stably for a couple of weeks. However the drying out problem should be fixed for the longer term stability.

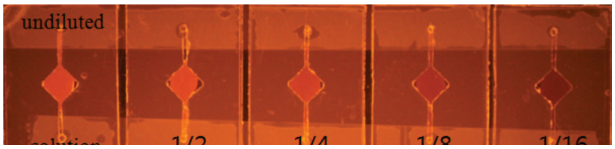

FIGURE 12. The reference plate image captured by the conventional 4 lamp UV illuminator

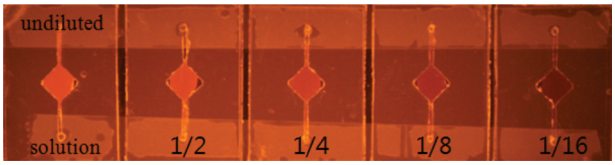

FIGURE 13. The reference plate image captured by the proposed UV illuminator

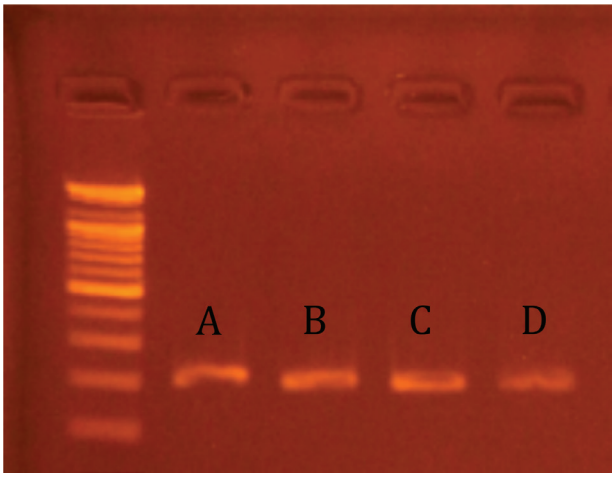

FIGURE 14. Chamber captured by conventional 4 lamps UV illuminator

CONCLUSION

We proposed the low cost UV illuminator with a single movable UV lamp to have the comparable performance with the conventional UV illuminator with 4 lamps. The DNA gel images from the proposed and the conventional UV illuminator were compared qualitatively and also quantitatively. The results showed that their performance is comparable. We also introduced the reference fluorescence plate with chambers filled with various dilutions of the colloidal quantum dots to eliminate the biochemical process to prepare DNA gels and their time-invariant emulation. The proposed reference plate well emulated the DNA gels and it was also utilized the comparison of the proposed and the conventional UV illuminators. The experimental results with the reference plate also showed the proposed UV illuminator perform similar to the conventional one. The reagent dry problem is currently being solved for the long term stability of the reference plate.

TABLE 4. Comparative analysis of the captured chamber

	undiluted solution	1/2	1/4	1/8	1/16
Conventional system	97	103	93	71	44
Proposed system	104	102	91	69	38
CV (%)	6.7	1.0	2.2	2.8	13.6

TABLE 5. The analysis of the captured Agarose gel

A	B	C	D	mean
98	101	99	84	95.5

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