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악성종양의 형광영상 진단을 위한 다파장 여기광원장치의 개발과 평가

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Development and Evaluation of Multi-Wavelength Excitation Light Source for Fluorescence Imaging to Diagnose Malignancies

Hyun Soo Lim

Department of Biomedical Engineering, College of Medicine ,Chungnam National University, Daejeon, Korea

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Abstract

This study aims at designing and evaluating light source devices that can stably generate light with various wavelengths in order to make possible PDD using a photosensitizer and diagnosis using auto-fluorescence. The light source was a Xenon lamp and filter wheel, composed of an optical output control through Iris and filters with several wavelength bands. It also makes the inducement of auto-fluorescence possible because it is designed to generate a wavelength band of 380-420nm, 430-480nm, and 480-560nm. The transmission part of the light source was developed to enhance the efficiency of light transmission. To evaluate this light source, the characteristics of light output and wavelength band were verified. To validate the capability of this device as PDD, the detection of auto-fluorescence using mouse models was performed.

Key words : PDD, fluorescence diagnosis, cancer, 5-ALA, PPIX, photosensitizer, Auto- fluorescence.

Ⅰ. INTRODUCTION

In Photodynamic Diagnosis (PDD), a photosensitizer injected into normal tissue is discharged from the organic body after a specific period, but is accumulated in abnormal tissues such as malignant tumor[1-3]. If the malignant tumor is then irradiated with light of the appropriate wavelength to cause electronic excitation of the photsensitizer, the generation of fluorescence by the interrelation of light and photosensitizer within abnormal tissues are observed. From the intensity and the spectrum of this light, the size of cancer and its boundary can be calculated. This diagnosis method has merit as a non-invasive method that does not result in damage of the body organs, different from the previous method of using a biopsy, because it uses fluorescence selectively generated from cancer cells. Also, PDD, helping group examination of lung cancer, makes possible the detection of lung cancer in early stage. The selection of the wavelength of excitation light has an interrelation with fluorescence generation used in the diagnosis of cancer, and the homogeneity of beam of excitation light is an important factor for PDD[4-6]. However, since injecting a photosensitizer can be a demerit, to complement this weak point, a method of auto-fluorescence using green fluorescence protein (GFP) and red fluorescence protein (RFP) such as NADH+ active factors within the organic body is developed. The previous light source developed for PDD cannot be applied to various fluorescence diagnoses because it generates a single wavelength. Therefore, this research developed an excitation light source that plays an important role in diagnosing cancer by PDD and is designed to provide multi-wavelength[7-9].

Corresponding Author : Hyun Soo Lim
대전광역시 중구 대사동 640
Tel : +82-42-280-7829 / Fax : +82-42-280-7829
E-mail : hslim@cnu.ac.kr
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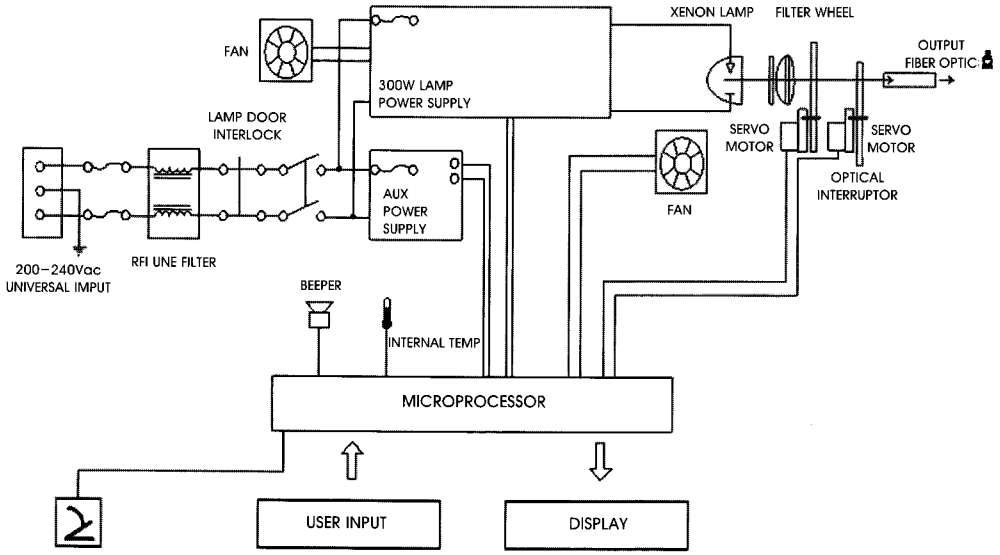

Fig. 1. Block diagram of excitation light source.

II. MATERIALS AND METHODS

A. System Configuration

For fluorescence diagnosis, sufficient outputs that can effectively excite GEP and RFP and wavelength bands of light correlating to this output are necessary [1, 2]. Figure 1 presents the interface of input/output of users, a main processing device controlling all sequences, a cooling system and a power supply device supporting the stabilized output of Xenon lamp, optical module controlling the intensity of output and wavelength of output light, and optical transmission device for the final output light.

1) Light Source and Design of Optical System

There is a difference in absorption coefficient according to the wavelength of each stratum of organic tissues, and the excitation wavelength differs according to fluorescence factors [5, 6]. As shown in figure 2, with Xenon lamp, which is the most similar light to natural light, wavelength of excitation light was selected.

Since Xenon Lamp includes the wavelength bands of visible ray, ultraviolet ray, and infrared ray, an IR filter (a hot mirror) and UV filter are used to block IR, including a large amount of thermal energy and harmful UV respectively. An IR filter protects optical fiber from thermal energy generated

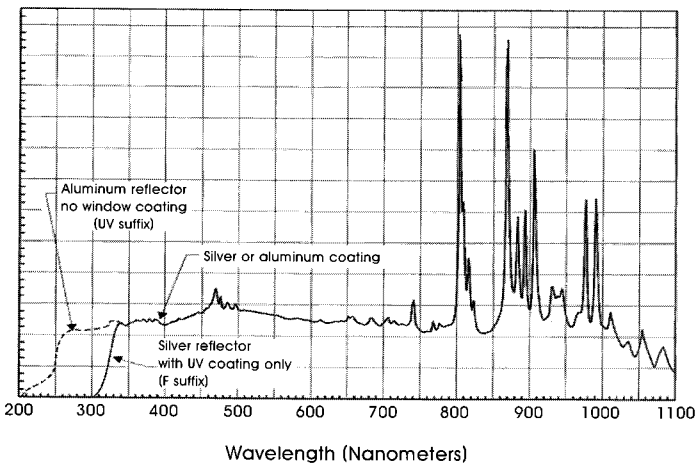

Fig. 2. Characteristics of optical output of the Xenon lamp.

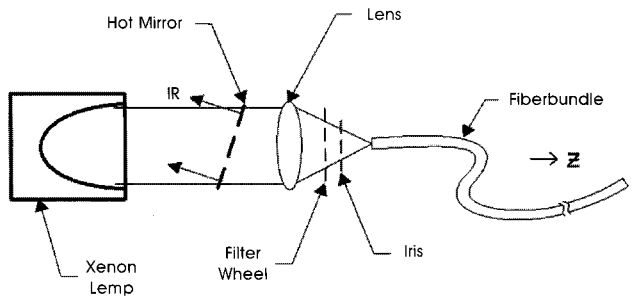

Fig. 3. Block diagram of the optical output and wavelength control parts.

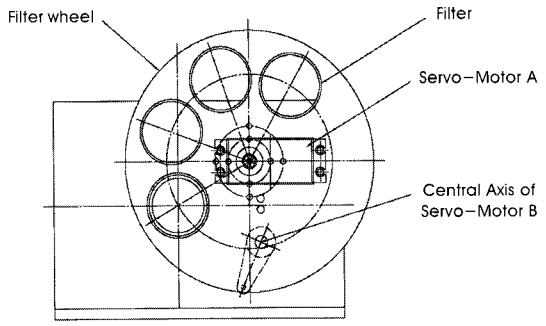

Fig. 5. Filter wheel and servo motor.

and an UV filter minimizes the damage of tissues caused by UV. It is essential to block UV because the excessive exposure of UV-A of 320-380nm causes death and mutation of cells resulting from the damage of DNA and membrane [7]. As shown in figure 3, among lights generated from Xenon Lamp, the effective optical wavelength band that passes through IR and UV filters is the visible ray area of 380nm-770nm. A band pass filter makes possible optical output of various wavelengths. An iris enables the control of quantity of light and a bundle of optical fiber is used to transmit output light effectively.

As shown in figure 4, an iris controlling the quantity of light was used to observe and regulate the output quantity of light effectively. The inner diameter was 25㎜(≒0.98inch). By controlling the caliber of iris through each motion of 0°-90°, the output quantity of light was regulated. The Iris consists of stainless steel that is not easily changed by the radiant light of Xenon Lamp. To prevent diffusion, non-gross paint was used. The location of the iris was 25㎜(≒0.98inch) from a condensing lens. The caliber that is transmitted to fiber-bundle through iris is from maximum 12.5㎜(≒0.48inch) to minimum 1㎜(0.04inch). As shown in figure 20, the rotary motion of servo motor was changed into each motion of iris.

Characteristics exited by each different wave according to organic tissues, protein, and characteristics of molecules must be considered. Because a filter structure that can generate light with various wavelengths is needed, the filter wheel seen in figure 5 was developed by using the identical light source, Xenon lamp, to output an appropriate wavelength of light to research objects.

A filter wheel equipped with an optical filter was designed to generate lights with various wavelength bands, which are indigo blue, blue, green, and white. A servo motor was used to control these lights. A filter of 25㎜(≒0.98inch) was located 17㎜(≒0.67inch) away from a condensing lens to prevent the diffusion of light, which comes from the lamp of the filter wheel.

A condensing lens plays a role in collecting light for light radiated from Xenon Lamp to be efficient for fiber optic, as shown in figure 6. Since the general fiber optic made of glass fiber is easily damaged by heat, it is located a bit more forward than optimum focus. In this study, a fused silica Plano convex lens with the focal distance of 50mm was equipped and fiber optic was located in 42㎜(≒1.66inch)

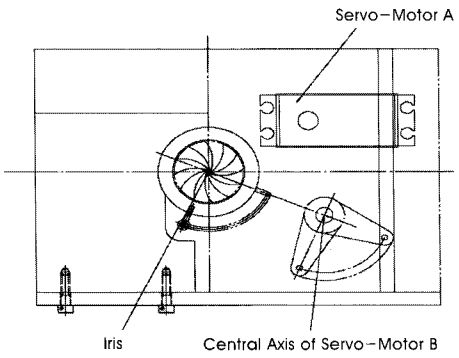

Fig. 4. Control part of quantity of light of Xenon lamp.

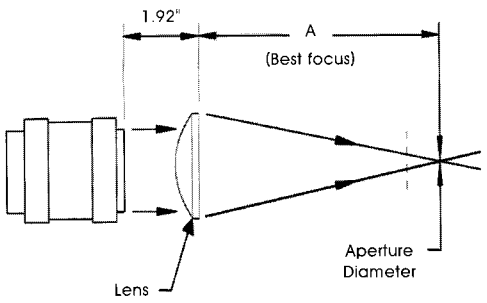

Fig. 6. Location of focusing lens and Xenon lamp.

2) Electricity Source of Xenon Lamp

To validate the electricity source of Xenon lamp, a supply device of electricity source was constituted. An electricity source of 14Vdc, 21A is needed for a Xenon Lamp to emit light. For the radiation, the arc inside the lamp in the early stage of the experiment was generated through a high voltage trigger of 30-45kV. After an electric discharge is kept by providing high voltage of 125-140Vdc made by boost-converter, which is connected to bundles of electricity source, 21A, a state of radiation is kept, through a stable electricity source by providing voltage of 14Vdc. All these processes are performed by a comparator and pulse circuit.

3) Temperature Control Part

For the stabilized light output of Xenon Lamp, it is necessary to keep a consistent temperature. The temperature is kept at 75℃, not exceeding a maximum of 150℃, which is a critical temperature of a lamp. By using a temperature sensor, temperature value was acquired. A temperature controlling device was embodied by speed control of fan through feedback.

4) Main Control System and Algorithm

A controlling part governs a motor used in the mechanical motion of the optical device and movement of a cooling system. It consists of a main controlling part and a user's interface as shown in figures 7 and 8. To protect a circuit from a spark of high voltage generated from an electricity source device of the lamp, inside the controlling board, a circuit of switcher voltage regulator was composed.

The microprocessor used in this study is the 80C196KC (20) from the Intel company. With characteristics of 80C196 KC, A/D convert of 8/10 bit is used to check the temperature of the lamp. A count of 16 bit and PWM are used to control the speed of a cooling fan. From these processes, the cooling system was embodied. The user's interface constitutes the user's 6 inputs and 26 action indications by enlarging input to

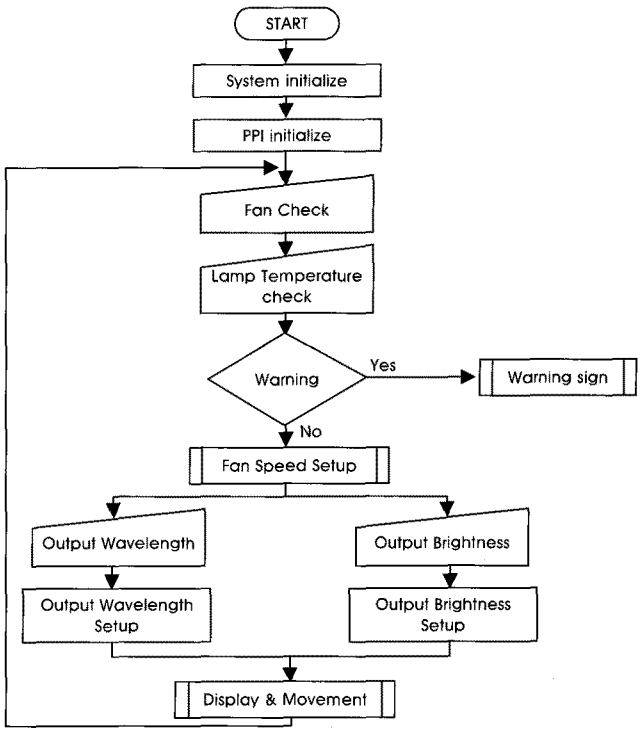

Fig. 7. Flow chart of the main program.

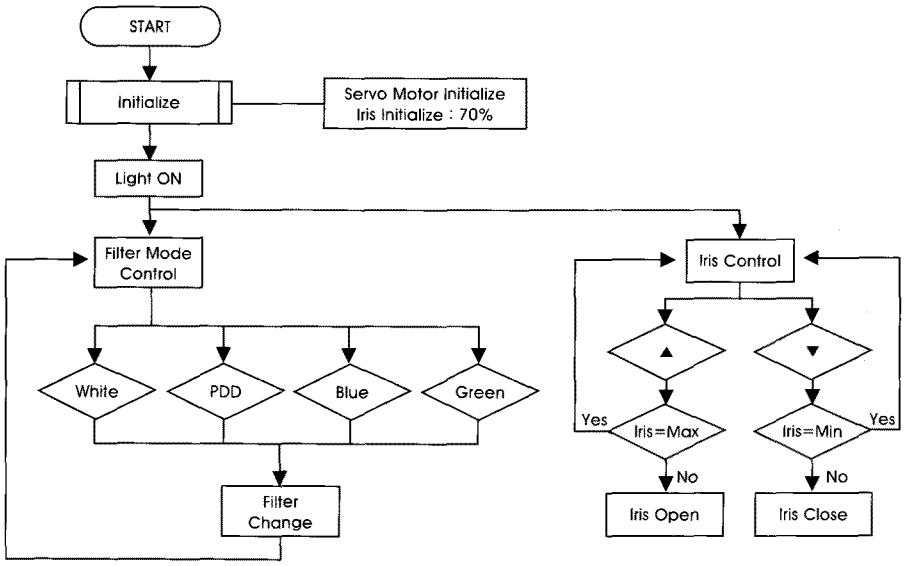

Fig. 8. Flow chart of wavelength selection.

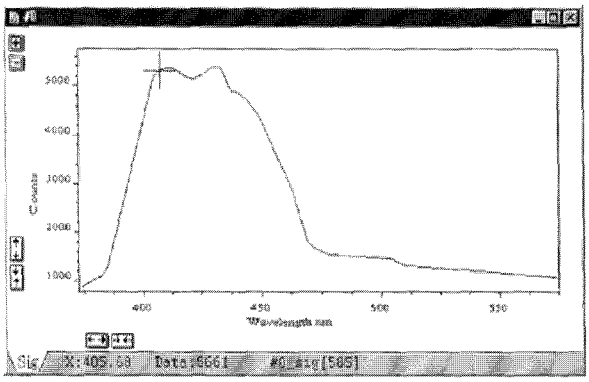

Fig. 9. Excitation light output of 400~480nm band.

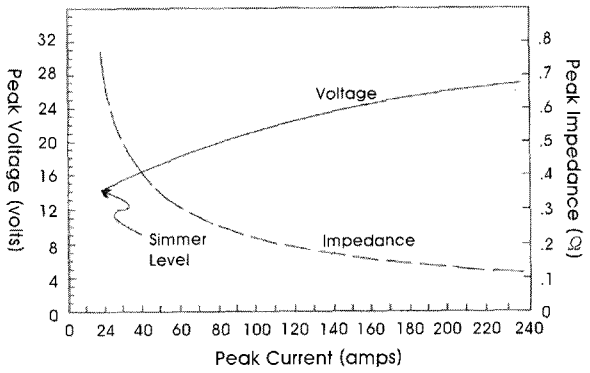

Fig. 11. Characteristics of electrical power of Xenon lamp's light source.

1 port and output 2 ports by 8255(Programmable Peripheral Interface: PPI).

Ⅲ. RESULTS

MS257 of Oriel instruments was used to calculate light output wavelength of the embodied light source device. WT210 Digital Power meter of Yokogawa and 544622D Oscilloscope of Agilent company were used to measure the characteristics of the power of light source electricity device.

The measure for the conduction of electromagnetic wave and electromagnetic wave radiation, which was consigned to the Korea Testing Laboratory, was performed by "Test method for prevention of electromagnetic wave hindrance" stated in Notification Number 1997-43 of the Ministry of Information and Communication.

Test of quantity of light, distribution of light, and color temperature was performed at an angle where luminance had a maximum output of 50% after the quantity of light was adjusted in the maximum output, fiber optical cable was connected into the exit and CL-200 illuminator of the Minolta company was located 5 cm away from the end of a fiber optic cable.

A. Performance Evaluation for Developed Hardware System

1) Wavelength band of 400-480nm

Figure 9 shows the result of measuring wavelength band of 400-450nm, which is an excitation light of ALA-5, a contrast medium. (Grove: 300)

2) Wavelength band of 380-420nm

Figure 10 shows the result of calculating an excitation light of 380-400nm, which is an excitation wavelength of auto-fluorescent factor (GFP). (Grove: 2400).

3) Measure of the characteristics of electric power of light source of Xenon lamp

Figure 11 schematizes the characteristics of electric power of the light source of the Xenon Lamp. Figure 12 presents a trigger pulse when the lamp turns on and characteristics of

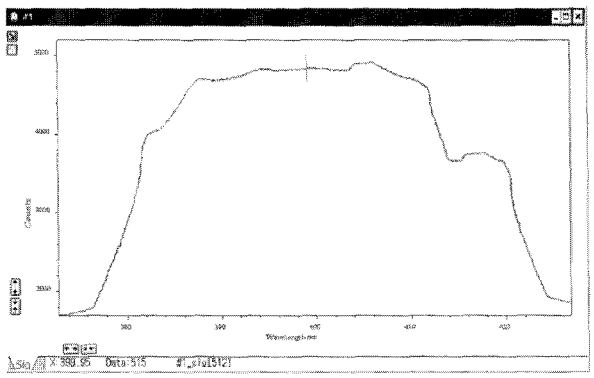

Fig. 10. Excitation light output of 380-420nm band.

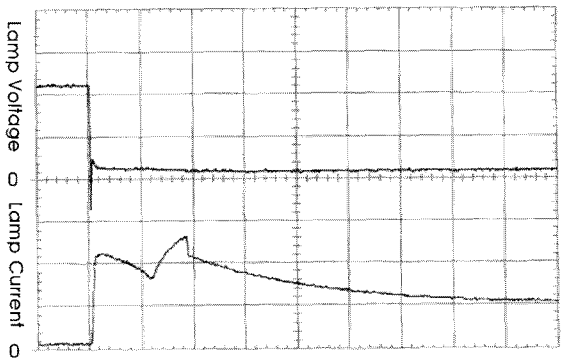

Fig. 12. Lamp trigger pulse.

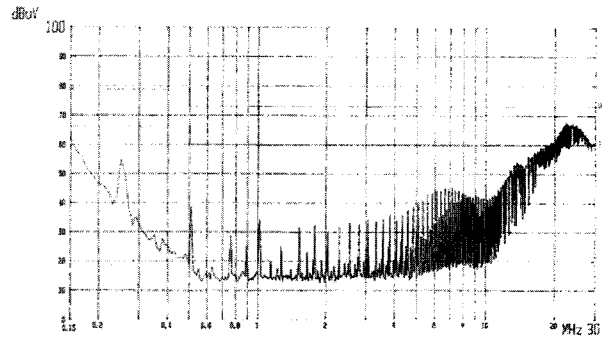

Fig. 13. Electromagnetic wave transmission (Live- Ground).

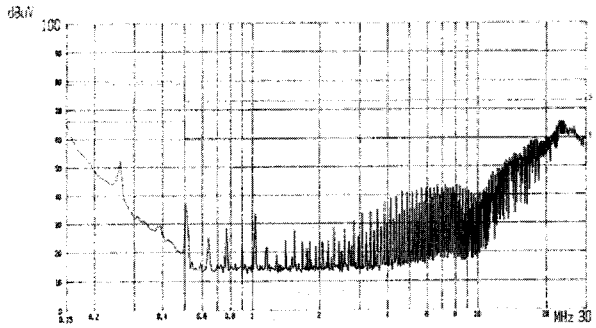

Fig. 14. Electromagnetic wave transmission (Neutral -Ground).

electric current and voltage while the lamp works, respectively.

4) Measure of Electromagnetic wave transmission and electromagnetic wave radiation

This test was performed by "Test method for prevention of electromagnetic wave hindrance" stated in notification number 2004-23 of the Ministry of Information and Communication and notification number 2004-30 of Radio Research Laboratory. This test satisfies the standard of these institutes, as shown in figures 13 and 14.

5) Electromagnetic wave radiation

The test results of electromagnetic wave transmission according to measured frequency, not exceeding critical value, satisfy the standard of electromagnetic wave transmission as to "Standard of prevention of electromagnetic wave hindrance for A grade device/the first class device", as shown in table 1.

6) Quantity of Light, Light Distribution, and Color temperature

Test of luminance, distribution of light, and color temperature was performed in an angle where luminance had a maximum output of 50% after the quantity of light was adjusted in the maximum output, a fiber optic cable was connected into the exit of light source and CL-200 illuminator of Minolta company was located in 5 cm away from the end of fiber optical cable, as shown in figure 15. Luminance was over 5.0×10^4 Lux, the distribution of light, over 45°± 5° and color temperature, 5600°K± 5% (Lamp used less than 500 hours)

B. Experimental Assessment of Fluorescence Radiation of Mouse model

The photosensitizer used in this research is Photogem, which is a product of the Moscow Institute of High Chemical Technologies. The object cell group to make tumor tissue was Lewis lung carcinoma cell. To cultivate these cells, DMEM

Table 1. Measurement of electromagnetic wave radiation

Test frequency (MHz)	Measured Value of Tester (dBμV)	Antenna Polarity	Compensation coefficient		Result value (dBμV/m)	Amount value (dBμV/m)	Margin
			Antenna factor	Cable loss			
44.7	15.4	V	137	0.8	29.9	40.0	10.1
56.7	23.8	V	13.0	1.0	37.8	40.0	2.2
92.2	28.1	H	9.6	1.2	38.9	40.0	1.1
131.8	24.2	H	12.9	1.5	38.6	40.0	1.4
214.0	11.0	v	10.3	1.8	23.1	40.0	16.9
230.0	15.0	V	11.2	1.9	28.1	40.0	11.9
308.7	30.3	V	13.0	2.2	45.5	47.0	1.5
425.7	27.9	V	15.4	2.6	45.9	47.0	1.1
866.4	17.2	H	21.2	3.8	42.2	47.0	4.8
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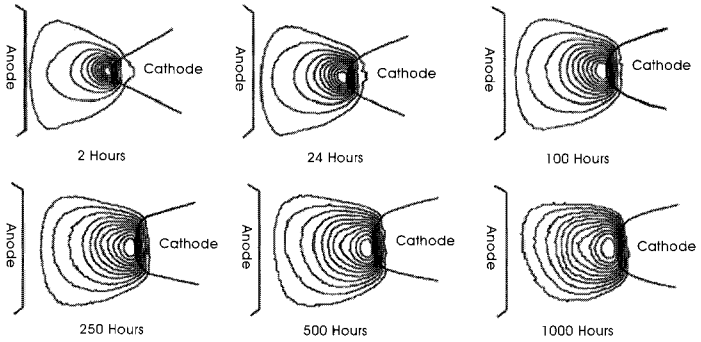


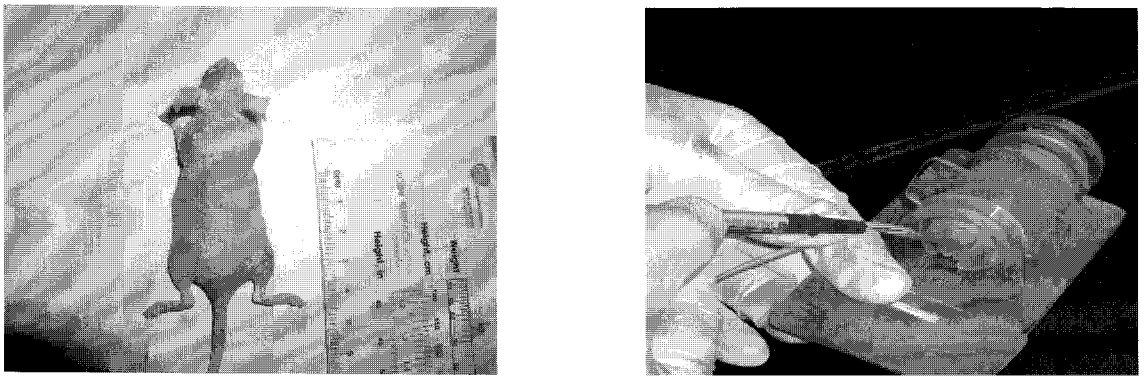
Fig. 15. Light distribution as to use time of a lamp.

media (Gibco, cat No. 12100-046) including FBS of 10% and bicarbonate (Sigma: S-5761) of 3.4g, and antibiotic-Antimycotic (Gibco, cat. No 15240-062) of 10ml were used. Eight hairless mice at the age of 6 weeks (Hoshino Laboratory Animals, Japan SLC, Inc), as shown in figure 16(a), were used for the clnical test. Drugs used for the transplantation of Lewis lung carcinoma cell and injection of Photogem were xylazine hydrochloride, ketamine and normal saline, whose portions are 1: 2: 9 respectively. Xylazine hydrochloride per mouse was 0.029mg, and ketamine was 1.442mg. After diluting cultivated Lewis lung carcinoma cells in trypsin or DMEM media, 0.1cc was injected in the femoral region of the left lower limb of a hairless mouse, to transplant cell numbers per each part 5×10^5 cells with a needle of 26G. On the seventh day after the tumor cells were transplanted, the size of tumor and the change of skin color were observed, and then only in the part where tumor was formed and touchable, the photodynamic diagnosis was performed. Figure 16(b) shows that Photogem was injected to the mouse 7 days after the Lewis lung carcinoma cell was transplanted. The way of giving photosensitizer is that photogem of 4mg/kg was diluted with saline solution of 1ml and 0.1cc was injected to the vein of a mouse's tail vein.

Figure 17 is an image after 12 hours of drug injection and shows that fluorescence is spread in the whole body of a mouse by extensive diffusion of photosensitizer. Figure 18 presents that drug is selectively deposited only in a part of malignant tumor after 17 hours of drug injection and fluorescent image is shown partly because it is emitted in a part of normal tissue.

Figure 19 shows the image after 20 hours of drug injection, where the fluorescence of the malignant tumor part is progressively clear but that of the normal part clearly disappears. Figure 20 presents an image 22 hours after drug injection, where there is a definite difference between the image of the malignant tumor and that of normal tissue.

Figure 21 shows a fluorescent image of tumor tissue cultivated in the femoral region of the left lower limb 24 hours after drug injection. The boundary between tumor tissue and normal tissue is distinctively presented.



(a) (b)

Fig. 16. Transplant of Lewis lung carcinoma cell transplant (a) and Photogem injection (b).

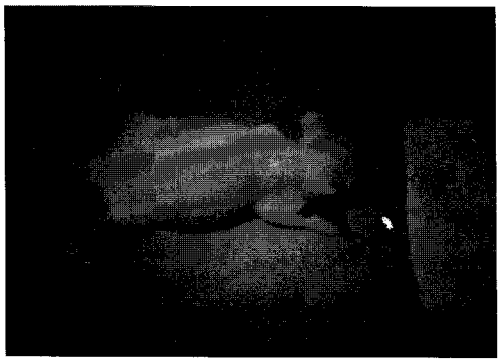


Fig. 17. Photosensitizer-induced fluorescence after 12 hours.

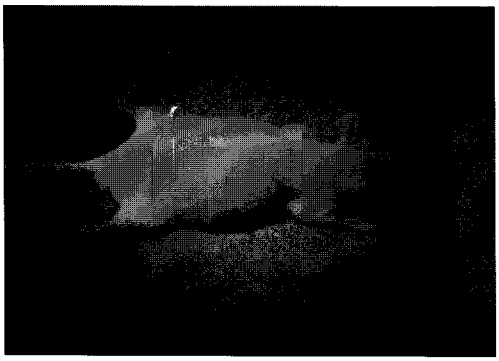


Fig. 18. Photosensitizer-induced fluorescence after 17 hours.

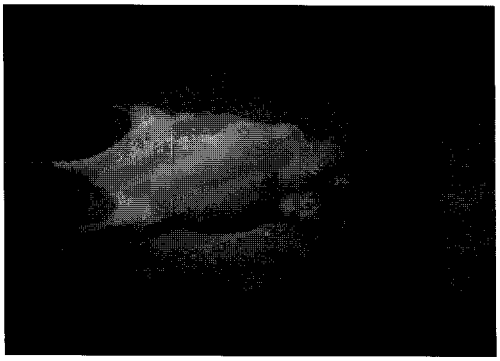


Fig. 19. Photosensitizer-induced fluorescence after 20 hours.

Ⅳ. DISCUSSION AND CONCLUSION

The purpose of this study is to implement an excitation light source for a fluorescence endoscope for photodynamic cancer diagnosis. The results satisfied the technical conditions and shows the light generation of multi-wavelength, light homogeneous. Trigger pulses when the lamp turns on are stable and electric current and voltage while the lamp works flow contant, respectively. The selection of wavelength band of excitation light according to photosensitizer was possible and

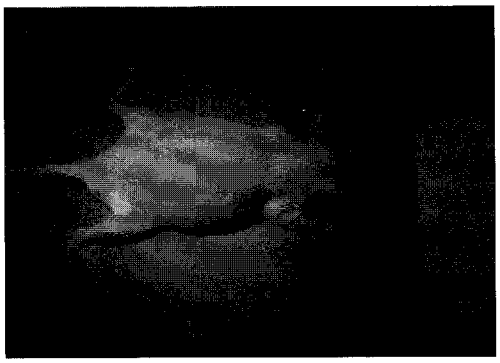


Fig. 20. Photosensitizer-induced fluorescence after 22 hours.

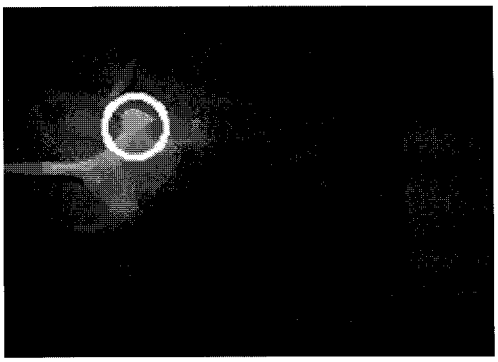


Fig. 21. Photosensitizer-induced fluorescence after 24 hours.

the experimental results show that power characteristics of the light source were excellent. Specifically, this test was perfo-rmed by "Test method for prevention of electromagnetic wave hindrance" stated in notification number 2004-23 of the Ministry of Information and Communication and notification number 2004-30 of Radio Research Laboratory. This test satisfies the standard of these institutes. The experimental assessment of mouse models by injection of fluorescence shows that as times passes, the intensity of fluorescence between normal tissue and tumor tissue is distinctively different. Especially, this study shows the possibility of fluorescence diagnosis by using GFP and RFP. Thus, further research on various photosensitizers, absorption characteristics of tissues of endogenic factors and optical absorption wavelength must be performed. Also, clinical application through clinical experiments must be evaluated.

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