Fluorescent property of indocyanine green (ICG) rubber ring using LED and laser light sources

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Abstract: Fluorescent properties of ICG depends on solvent. Fluorescent characteristics of ICG rubber rings and optimized detection system condition were identified. The fluorescent rubber rings are produced by drying mixture of ICG solution and liquid rubber. LED and laser light sources were used to test differences between them. Other variables are ICG molar concentration (100, 80, 60, 40, 20, 10 μM), excitation lights (740, 760, 785nm) and angle of view (0~80°). We observed that ICG ring emitted fluorescence at longer wavelength than in blood and aqueous state. Observation angle between 0 and 50 provided similar brightness of images, while others are significantly less luminous. Excitation light between 740–760nm ensured non-overlapping spectrums of excitation light and fluorescence emission.

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1. Introduction

Use of fluorescence material in surgery is becoming popular as they can mark tumor and blood vessel allowing more effective and safe surgery [1,2]. Many of fluorescence imaging methods and materials are currently under research.

Indocyanine Green, known as ICG, has been approved of clinical usage since 1956 [3,4], used dominantly in angiography from about ten years after its approval [5]. ICG’s long clinical usage verified many advantageous characteristics such as safety, good SNR (signal to noise ratio), short lifetime and cheapness [6]. Recently, ICG imaging is broadening its application to cancer treatment, reconstructive surgery, laparoscopy [7,8] and other areas [9].

In colorectal surgery, resecting 5 cm of normal tissues on either side of tumor is optimal [10]. To ensure minimally invasive surgery and complete resection of tumor, an endoscopic fluorescent band ligation method was recently proposed by Hyun et al [11]. Tattooing with ICG or India ink and endoscopic clips were traditionally used to localize gastrointestinal tumor. ICG solution and liquid rubber mixture is made into fluorescence rubber rings to mark tumor during colorectal resection surgery. However, they have severe drawbacks such as faint or fuzzy tattooing and invisibility of the clip outside the bowel. Several ICG rings are clipped on mucosae around the tumor through endoscope and observed during laparoscopic stomach and colon surgery through fluorescence laparoscope. Thus, use of ICG rubber ring can provide visual information of tumor location to the operator, ensuring complete and effective resection of tumor.

ICG’s fluorescence properties vary with the type of solvent, excitation light spectra, filter and ICG concentration [12]. Its properties in blood and aqueous solution are thoroughly investigated by many studies. ICG absorbs red light about 740–800nm [13–16] and emits fluorescent light peaking at 830nm in blood and 820nm in aqueous solution [17–19]. The
quantum efficiency of ICG is low, 0.3% in water and 1.2% in blood [20]. It emitted most intense fluorescence light at 40μM in triple distilled water under 740nm light source [21].

However, ICG’s fluorescence properties in rubber solvent are not well known. For optimized use of ICG rubber ring in clinical situation, information of its fluorescence properties is essential since ICG’s fluorescence properties change with its environment [22,23]. Variables affecting observed fluorescence images such as filter, excitation light’s spectrum, and angle of view should also be known to obtain optimal condition for ICG rubber ring detection.

Furthermore, laser and LED have different spectral properties and benefits from each other. This study compared the laser and LED light sources in terms of their effect on the fluorescent property of ICG rubber ring. Laser had a narrower range of light wavelength when compared to LED although shifting of peak light wavelength was observable due to heating.

In this study, rubber rings with various concentration of ICG were observed by spectrometer and experimental surgical microscope with NIR camera under LED and laser light sources. The result was analyzed to obtain concentration that has the best fluorescent response and to assess the effect of different light source and range on ICG’s peak fluorescent wavelength and intensity. Spectrometer was used to measure peak wavelength and intensity of ICG fluorescence making comparison between the LED and laser light sources. LED and laser Experimental conditions, such as angle of view and distance between ICG ring and observer instrument, were altered to mimic the environment during laparoscopic surgery or others.

2. Materials and methods

2.1 ICG sample preparation

25mg of ICG and 10ml of aqueous solvent were obtained from Daiichi Sankyo Company, Limited. ICG solution of 3.5mM was made first and then it was diluted to 6 different concentrations (10, 20, 40, 60, 80 and 100μM) by liquid rubber solution from Unidus in Korea. Since its fluorescence peak was at 40μM in triple distilled water, 10, 20, 40, 60, 80 and 100μM ICG rubber solution were made. This solution was then put into a mold (Fig. 1(a)) and dried to make ICG rubber rings (Fig. 1(b)).

![Fig. 1. ICG rubber solution (a) in a ring mold, (b) ICG rubber ring, increasing in concentration from left (10μM).](image)

2.2 Experimental setup

In this study, two types of fluorescence apparatus were set up to obtain spectral information and visual image of the ICG rubber rings, and they are shown in Fig. 2. Figure 2(a) shows
schematic illustration of the setup using spectrometer (Jaz, Ocean Optics, USA) and LED (ARISTO Microscope Light, MG Bendoscopy, Korea) in Fig. 2(b) and laser light source of 760nm (ADR-1805, Leading-Tech Laser, China) and 785nm (MDL-III=785nm, Chanhum New Industries Optoelectronics Tech, China) in Fig. 2(c). All lasers were used with cooling system that was provided by the manufacturer. Additional cooling system was not used since in this experiment and in clinics, lasers are only used for a short period of time. Since the apparatus aims to get spectrum graph of absorption and emission light as in condition similar to laparoscopic surgery, light source and spectrometer were placed in the same axis.

740nm LED lamp was specially designed to focus light at about 20cm away, thus the lamp was placed 20cm away from the ICG rubber ring (13.67mW/cm²). Laser light sources (760nm, 785nm) were placed about 1cm away from the ICG rubber ring due to their small focus and rapid loss of intensity with distance (55mW/cm²).

Various concentrations of ICG rubber rings were placed under the two apparatus under the same condition and the information was recorded through exporting the spectrum graph values to excel and capturing images. However, when conducting the experiment for the effect of angle of view, the light source remained vertical and spectrometer tilted at an angle.

2.3 Information gathering

Spectrum graph from the spectrometer was adjusted through software so that the intensity curves of the light source and ICG fluorescence can fit within the maximum range of the spectrometer (65535 counts). Software adjustment of integration time at 11ms ensured it.

Figure 3 shows features of surgical microscope (Opmi nc, Zeiss, Germany) used to gain visual images of the ICG rubber ring. The light from the source and to the CCD camera (BU406M, Toshiba Teli Corporation, Japan) exists on the same plane as in laparoscope. Due to problems listed before with the laser light source, only 740nm LED light source was used in surgical microscope. In order compensate for light attenuation from beam splitter and to maximize the sensitivity, wide bandpass filter (832nm CWL, 25mm Dia, 37nm Bandwidth, OD 6 Fluorescence Filter, Edmund Optics, USA) with 90% transmission was used.
Images were taken at frame rate of 30 fps with exposure time of 31300μs which was maximum value for the set frame rate and it was sensitive enough to show differences when variable was changed and robust. All experiments were conducted in room temperature and conducted within 5 days of ICG rubber ring production before ICG fluorescence starts to fade.

Quantum efficiency can be measured through the ratio of photons absorbed and emitted, or making a relative comparison to sodium fluorescein [20]. In this experiment quantum efficiency (Q.E.) of ICG rubber ring is measured through the intensity ratio of the peak wavelength of excitation (excitation) and emission lights (fluorescence) shown in Eq. (1).

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Q.E.(%) = \frac{\text{fluorescence}}{\text{excitation}} \times 100\% \tag{1}
\]

3. Results

When different concentration was observed under 740 nm LED excitation light source, ratio of absorption to emission decreased with increasing molar concentration as seen in Fig. 4 and each concentration is measured 4 times. This trend was different in 760nm and 785nm laser light. Unusually high ratio was recorded under 760 nm light at ICG concentration of 60μM. In 785nm laser source, 40μM ICG rubber ring had the highest ratio.
Fluorescence emission from ICG depends on many variables and the relationship between concentration and fluorescence behavior of ICG was investigated to assist setting optimal detection system condition in the future research. Concentration and source light affected the peak fluorescence wavelength from the ICG rubber ring ranging from 828nm to 871nm (Fig. 5). Generally, the longest wavelength of peak fluorescence emission wavelength was recorded between 60μM and 80μM in all excitation light source spectra. 100μM in 760nm source light and 80μM and 100μM in 785nm light source were omitted as they were indistinguishable under the same condition.
Figure 5 shows spectrum graph of source light and ICG fluorescence measured through spectrometer for 20μM ICG rubber ring which was the most fluorescent out of other concentrations. As it can be inferred from Fig. 6, fluorescence spectrum accounts for small percentage compared to the light source, the integration time for the graph was set to 100ms exceptionally (other figures are with 11 ms) to show fluorescence peaks. Fluorescence emission and excitation light source spectrum did not overlap under 740nm and 760nm light sources having distinct peaks. 785nm light source had two peaks overlapping thus hard to know spectrum graph of fluorescence. Non-overlapping spectrums are important in obtaining reliable image of ICG rubber ring free from interference of excitation light, especially when fluorescence emission intensity is significantly lower than excitation.
Depending on the insertion angle of laparoscope in surgery, the image of ICG rubber ring observed by the operator may vary. Figure 7 and Fig. 8 shows effect of change in angle of view on the image gathered by CCD camera. The light source and CCD camera were fixed in same axis and when they were orthogonal to the plane on which the ICG rubber ring is place, it was 0° and angle between them was increased (becomes more parallel to the plane) until 80° in 10° interval. As more top surface of the ring was seen, image had higher average pixel value. Visual images (30fps at 31300μs exposure time) of this data are shown in Fig. 8.
Fig. 7. Average pixel values of images through CCD camera with varied angles under 740nm excitation light source. n=3.

Fig. 8. Images taken from CCD camera when angle of view was changed from 0° (left) to 80° (right).

Figure 9 shows the average pixel values of ICG ring observed through the CCD camera with bandpass filter when the distance between the ring and camera were changed. LED with light spectra peaking at 740nm was used and it was specially designed have focus at about 20cm in front. Regardless of the distance, 20μM was the brightest and the general trend of decreasing image pixel intensity as the ICG concentration deviates from 20μM. Closer the excitation light was placed to the ICG rubber ring, brighter image was obtained.

Fig. 9. Average pixel values of images when the distance between the ICG rubber ring and excitation light (740nm LED) were changed (150, 200, 250mm). n=3.

4. Discussion

The quality of the image gathered by CCD camera heavily depends on the filter, selecting range of light wavelength, the intensity of light reaching CCD camera and the sensitivity of CCD camera itself. This study aimed to provide fundamental fluorescent properties of ICG in rubber solution to maximize the quality of image through CCD camera through aiding future research with the choice of filter to be used and other experimental conditions.

LED and laser light sources did not have effect on the fluorescence properties of ICG rubber ring. In both LED and laser light sources, ratio of absorption to emission was between...
1.2–1.6% range for 10μM and 20μM which were the brightest concentration under camera observation. This value was sufficient for observing the ICG rubber ring clearly.

Laser light source apparently had narrower range of light emitted than the LED thus better able to separate fluorescent and excitation spectrum as there is less overlapping area between them. Yet the problem was peak wavelength shift due to heating and small area under light.

Since the rubber dries from liquid state to solid, the actual concentration of ICG in rubber is expected to be higher than when it was measured in liquid solution state. Thus the brightest concentration of ICG rubber ring is around 20μM in liquid solution state, so in dried state, the concentration is expected to be higher and perhaps similar to the value in aqueous state (40μM). However, this needs to be verified in the future research. Trend in ratio of absorption to emission was different with the excitation light used due to the different absorption spectrum of the ICG rubber ring. Generally, as ICG concentration increases, less fluorescence light is emitted compared to light absorbed appearing darker in the image. This may be mainly due to quenching effect [24]. A higher ICG concentration, dimers and polymers of ICG are formed emitting fluorescence at shorter wavelength. This leads to decrease in yield and appear darker in the image. Absorption spectra of the ICG much depend on the concentration.

740nm and 760nm excitation lights are suit for accurate detection of ICG fluorescence since the spectrums of excitation light and fluorescence emission did not overlap. Non-overlapping spectrums are important when gathering the image from the camera as excitation light may influence the image if spectrums overlap, indicating that the image is not a perfect representation of the fluorescence emission. Further study is needed to confirm the actual range of appropriate excitation light source wavelength since only 3 different ranges of light are tested in this study.

Variation in observed pixel values with angle of view was due to the shape of the ring. Since the light source and CCD camera were in fixed relative position, scattering due to curved surface and spreading of the light reaching the angled surface were the reasons for decreasing pixel values as the angle of view gets more tilted sideways (horizontal). Image was brightest when the ICG rubber ring was viewed directly from the top (vertical). Thus, it is crucial to maximize surface area of the ICG ring to gather most fluorescence from the ICG rubber ring.

Intensity or distance from the ICG rubber ring had a simple relationship. The LED used in this study is specially designed to concentrate light at 20cm, but the result shows that this focusing of light was not enough to overcome the intensity increase from simply putting the light closer to the ICG rubber ring. Brightness of the image increased when the intensity of the excitation light was increased, thus intensity of the source light should be adjusted to suit camera condition (exposure time, frame rate etc.).

In this study, we noted that the ICG in rubber had life-time much longer than in blood but its fluorescence property faded slowly in several days. This process was fastened if the ring was exposed to near infrared light of its excitation wavelength. Therefore, further research is need to know ICG life-time in rubber ring state as it is linked to its shelf life.

5. Conclusion

In this study, it was confirmed that the fluorescent properties of ICG in rubber solution were different to those of aqueous or blood. When the ICG is observed under a surgical condition varying peak fluorescence emission wavelength according to the angle of view should be accounted to maximize the accuracy of the presented image through the camera. Also, any other factors that might influence the quality of image were tested and this information could be used in the future research to obtain better fluorescent images of ICG through camera.
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