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ORIGINAL ARTICLE

# A method to identify pulmonary intersegmental planes with intravenous vitamin $B_2$ injection

Daisuke Saito[1] · Isao Matsumoto[1] · Ryuichi Waseda[2] · Keisuke Tatemichi[1] · Yusuke Tanaka[1] · Shuhei Yoshida[1] · Munehisa Takata[1] · Masaya Tamura[1] · Hirofumi Takemura[1]

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## Abstract

**Purpose** The present study investigated whether the pulmonary intersegmental planes could be identified with the intravenous injection of vitamin $B_2$ using a fluorescent camera and whether this method can be used instead of the inflation–deflation technique or the intravenous indocyanine green (ICG) method.

**Methods** In experiment 1, the vitamin $B_2$ was intravenously injected to visualize the pulmonary intersegmental plane and perform segmentectomy, and the visualized pulmonary intersegmental line was then compared to the inflation–deflation line in six pigs. In experiment 2, using six pigs, the fluorescent area and duration of fluorescence were compared after the intravenous injection of vitamin $B_2$ and ICG in the same animals.

**Results** In all animals in experiment 1, it was possible to clearly detect yellow–green fluorescence in the lung, in segments other than the one intended for resection, for at least 60 min. Moreover, the line visualized with vitamin $B_2$ fluorescence matched the inflation–deflation line in all animals. In experiment 2, the area of vitamin $B_2$ fluorescence corresponded to the area of ICG fluorescence in each animal.

**Conclusions** The visualization of fluorescence after the intravenous injection of vitamin $B_2$ using a fluorescent camera was a simple, safe, and accurate method for detecting intersegmental planes in a pig model. This method can be an alternative to the inflation–deflation technique and the intravenous ICG method.

**Keywords** Vitamin B2 · Lung segmentectomy · Intersegmental plane · Fluorescence · Non-small cell lung cancer

## Introduction

Precise identification of the pulmonary intersegmental plane is needed for optimal segmentectomy. An optimal method to identify the pulmonary intersegmental planes would accurately and clearly delineate the pulmonary intersegmental plane both at the lung surface and in the lung parenchyma, regardless of the surgical approach or underlying pulmonary conditions.

Various methods to identify pulmonary intersegmental planes have been reported [1]. Classically, many surgeons have used the intersegmental pulmonary vein as a guide [2]. In addition, surgeons currently use the "inflation–deflation technique," a method that is commonly used to identify the pulmonary intersegmental planes by creating an inflation–deflation line on the visceral pleura [3]. However, in patients with underlying pulmonary conditions such as emphysema, it is often challenging to accurately identify the pulmonary intersegmental planes.

As Matsumura et al. [4] reported, techniques to visualize the pulmonary intersegmental planes using fluorescent substances have recently been developed [5, 6]. In Japan in particular, visualization with intravenous indocyanine green (ICG) using pulmonary artery blood flow [7] is in use (hereinafter, this is called "the intravenous ICG method"). However, one of the disadvantages of the intravenous ICG method is that the duration of fluorescence visualization is short. Moreover, ICG is associated with the risk of allergic

✉ Isao Matsumoto
mat@p2223.nsk.ne.jp

[1] Department of Thoracic, Cardiovascular and General Surgery, Kanazawa University, 13-1, Takaramachi, Kanazawa, Ishikawa 920-8641, Japan

[2] Department of General Thoracic, Breast and Pediatric Surgery, Faculty of Medicine, Fukuoka University, 7-45-1, Nanakuma, Jonan, Fukuoka 814-0180, Japan

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reactions and tissue toxicity. For these reasons, a novel method that safely and accurately identifies pulmonary intersegmental planes is needed.

We have been developing a thoracic surgery procedure that uses vitamin $B_2$ for fluorescence navigation. We have reported the usefulness and safety of vitamin $B_2$ for identifying pulmonary sentinel lymph nodes [8]. Moreover, we have demonstrated, both in vivo [9] and in vitro [10], that it is possible to identify pulmonary intersegmental planes using a fluorescent camera following the transbronchial administration of vitamin $B_2$. However, the inflated lungs with the transbronchial administration of the vitamin $B_2$ solution obstructed the visibility of the operative field in video-assisted thoracoscopic surgery (VATS). We have, therefore, devised a method to identify the pulmonary intersegmental planes with the intravenous injection of vitamin $B_2$, taking advantage of the pulmonary artery blood flow.

The purpose of this study was to determine whether it is possible to identify the pulmonary intersegmental planes with the intravenous injection of vitamin $B_2$ using a fluorescent camera and to investigate whether this method can be used as an alternative to the inflation–deflation technique and the intravenous ICG method.

## Materials and methods

This study consisted of two experiments. In experiment 1, intravenous vitamin $B_2$ was used to visualize the pulmonary intersegmental plane and perform segmentectomy, and the visualized pulmonary intersegmental line was compared to the pulmonary intersegmental line determined using the inflation–deflation technique. In experiment 2, the fluorescent area and the duration of fluorescence after vitamin $B_2$ and ICG injections were compared and evaluated in the same animal.

The animal experiments in this study were conducted in accordance with the US Institute of Laboratory Animal Resources (ILAR) Guide for the Care and Use of Laboratory Animals [11] and with the Kanazawa University Animal Experimentation Regulations (approval no. 143240) stipulated by Kanazawa University.

### Fluorescent camera for vitamin $B_2$ visualization

An endoscopy system for photodynamic diagnosis (PDD; TRICAM ENDOSCOPE/D-light system®; KARL STORZ GmbH & Co., Tuttlingen, Germany), was used as the fluorescent camera. This endoscopy system excites photosensitizers, such as porphyrin compounds, by irradiating them with a special light and selectively diagnoses the neoplastic site [12]. By exchanging the filter, this system can detect normal light, autofluorescence, and extrinsic fluorescence. With an excitation light of 375–445 nm, extrinsic fluorescence with a 480–800 nm wavelength can be observed. In this study, excitation light was irradiated onto vitamin $B_2$ with the D-Light system®, and extrinsic fluorescence was detected and visualized with a TRICAM ENDOSCOPE®.

### Infrared thoracoscopy for ICG

The NIR/ICG system (KARL STORZ GmbH and Co.) was used as the ICG fluorescence system. This system uses light with near infrared wavelengths to enable the visualization of areas lacking ICG perfusion.

### Fluorescent substance

Vitamin $B_2$, a yellow water-soluble vitamin, was used as the fluorescent substance. It is theoretically a non-allergenic and non-toxic vitamin that emits 550-nm yellow–green fluorescence with an excitation light with a wavelength of approximately 450 nm. In humans, vitamin $B_2$ is converted to flavin mononucleotide and flavin adenine dinucleotide (FAD) through cellular enzymatic actions, with most becoming FAD sodium [13]. FAD sodium (Flavitan injection; TOA EIYO Co., Ltd., Fukushima, Japan) was used in this study.

### Anesthesia technique

Ketamine (10 mg/kg; Daiichi Sankyo Propharma, Tokyo, Japan) was intramuscularly injected to induce anesthesia in adult pigs. Vascular access through a peripheral venous route was obtained at the auricular vein. An intratracheal tube (24 Fr; Fuji Systems Co., Tokyo, Japan) was inserted after tracheotomy. Anesthesia was maintained with isoflurane (0.5–1.5%; Pfizer Japan, Tokyo, Japan) inhalation anesthesia, and muscle relaxation was achieved with vecuronium (0.1 mg/kg; Fuji Pharma Co., Ltd., Tokyo, Japan). A bronchial blocker (COOPDECH®, Daiken Medical Co., Ltd., Osaka, Japan) was used to perform unilateral lung inflation.

### Pig lung anatomy

The anatomical structure of the pig lungs has been described in detail [14]. According to Nakakuki et al. [14], pig lungs consist of dorsal, lateral, ventral, and medial bronchial systems. Pulmonary intersegmental planes, which are present between the pulmonary segments, are composed of loose connective tissue in pigs and are therefore easier to macroscopically visualize in comparison to the human lungs. Conversely, pig lungs have poor intersegmental pulmonary venous vasculature in comparison to human lungs. In our previous reports on identifying pulmonary intersegmental planes through the transbronchial administration of vitamin $B_2$ [7, 8], we used the right lungs of pigs. Because the right

pulmonary artery branching is somewhat complex in pigs, we used the left lung in the present study to use the pulmonary artery blood flow. The left lungs of pigs consist of the cranial lobe and caudal lobe.

The left cranial lobe consists of the cranial segment and caudal segment, and a pulmonary intersegmental plane is present between the two segments. In this study, segmentectomy of the cranial segment of the cranial lobe was performed. This segmentectomy corresponds to left upper division segmentectomy, a common procedure performed in humans.

## Experiment 1

### Pulmonary segmentectomy in pig lungs

In the right lateral decubitus position, lateral thoracotomy was performed at the fourth intercostal space on the left side. The cranial vein was taped without a dissection procedure (Fig. 1a). The cranial artery was identified (Fig. 1a). The cranial bronchus was identified between two vessels (Fig. 1a). After ligating and dissecting the pulmonary artery and bronchus that supply the identified cranial segment of the cranial lobe (Fig. 1a), vitamin $B_2$ (200 mg/body) was infused from the auricular vein.

Observation with a fluorescent camera showed that the pleura of the lung was divided into a fluorescent area and a non-fluorescent area. The changes in fluorescence were observed and recorded for 60 min after vitamin $B_2$ injection. To investigate the difference between the present method and the inflation–deflation technique, an inflation–deflation line was created with temporary bilateral lung inflation 15 min after an intravenous injection of vitamin $B_2$. Then, 30 min after the intravenous injection, the visceral pleura between the fluorescent area and the non-fluorescent area was marked with an electric scalpel (Fig. 1b). Next, while verifying the fluorescent area and the non-fluorescent area in the lung parenchyma, the intersegment was dissected with scissors (Fig. 1c). After dissecting the pulmonary vein, segmentectomy of the cranial segment of the cranial lobe was concluded (Fig. 1d). Subsequently, the remaining lung was inflated to perform a sealing test.

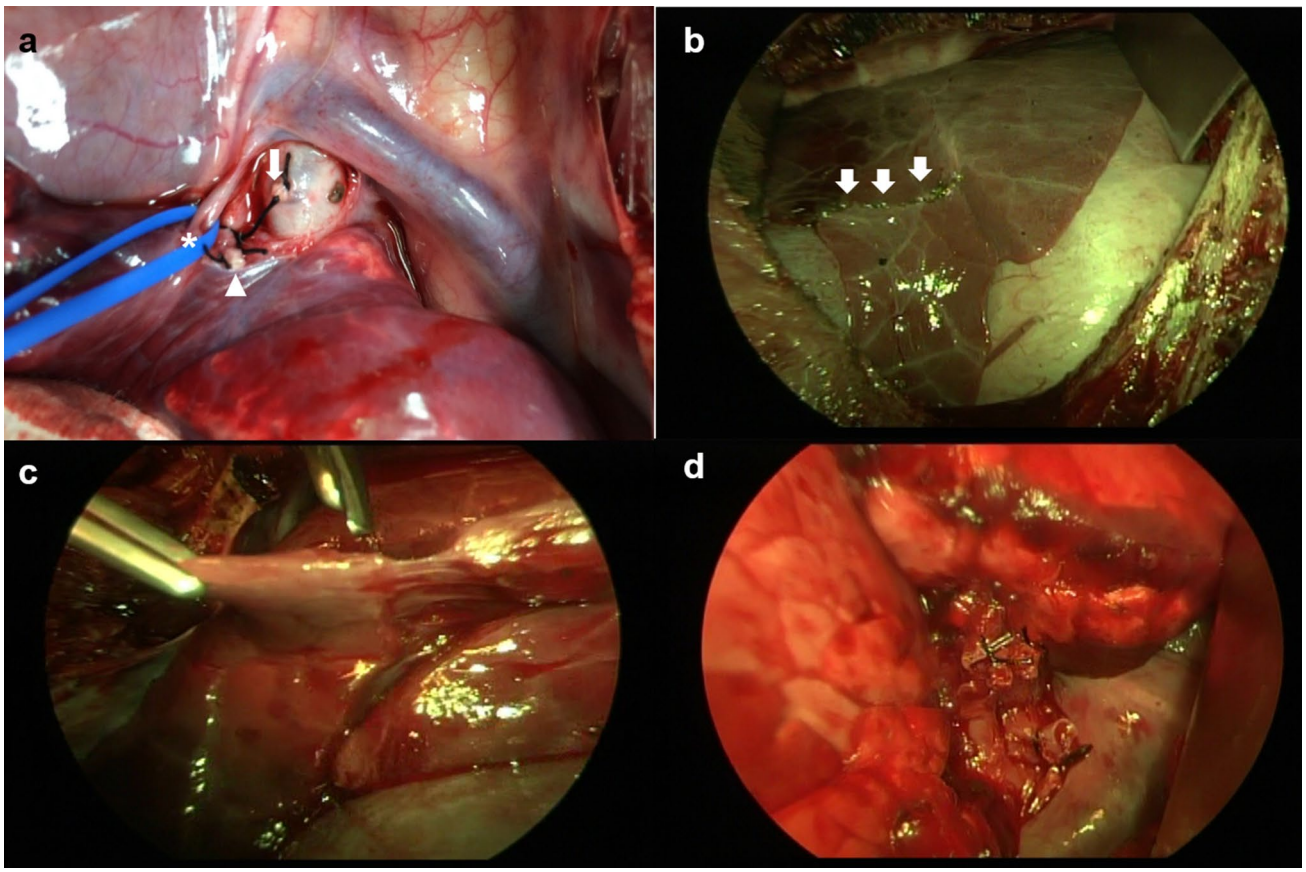


**Fig. 1** Intraoperative findings. **a** The cranial segment of the cranial lobe after processing the pulmonary artery and bronchus (arrowhead, cranial bronchus; arrow, cranial artery; asterisk, cranial vein). **b** The intersegmental plane is marked with an electric scalpel (arrow). **c** Dissection of the intersegmental lung parenchyma. **d** Left cranial lobe cranial segmentectomy

### Data collection and measurements

The durations of fluorescence visualization and luminance at the fluorescent and non-fluorescent areas after the administration of vitamin $B_2$ were measured. In addition, the accuracy of the pulmonary intersegmental plane visualized with vitamin $B_2$ was evaluated.

A luminescence-analysis software program (U11437, Hamamatsu Photonics; Hamamatsu, Japan) was used to analyze the luminance of the fluorescent area and the non-fluorescent area. At 0, 5, 10, 20, 30, and 60 min after the intravenous injection of vitamin $B_2$, the luminance at the fluorescent area and non-fluorescent area was quantitatively measured. Luminance is determined based on the weighted average of the RGB value and is expressed as an integer from 0 to 255 with a combination of red, green, and blue values (0, black; 255, white).

To assess whether the dissected surface is indeed the intersegmental plane, the intersegmental plane was verified by observation by the naked eye, and the absence/presence of bronchial damage at the dissected surface was determined. Air leaks from the dissected surface of the segment were also evaluated with a sealing test. Air leakage was rated from 0 to 3 according to the Macchiarini scale [15], as follows: 0, no leakage; 1, countable bubbles; 2, stream of bubbles; and 3, coalesced bubbles. Whether the pulmonary intersegmental line visualized with vitamin $B_2$ fluorescence corresponded to the inflation–deflation line that was created intraoperatively was examined with a video recording.

## Experiment 2

### Comparison with ICG

In the same adult pig, the extent of fluorescence in the segments was compared between vitamin $B_2$ and ICG. Similar to the segmentectomy procedure in experiment 1, the pulmonary artery and bronchus in the cranial segment of the cranial lobe were ligated, and 5 mg of ICG (Daiichi Sankyo Propharma, Tokyo, Japan) was intravenously injected. Using the ICG fluorescent system, the intersegmental line at the lung surface was observed and recorded. After fluorescence quenching of ICG, vitamin $B_2$ was administered intravenously to the same animal, and the fluorescence was recorded for a maximum of 1 h after injection. The video recording was used to determine whether the area of ICG fluorescence corresponded to the area of vitamin $B_2$ fluorescence.

### Data collection and measurements

After the intravenous injection of ICG and vitamin $B_2$, the time during which the intersegmental line was visible was measured. Additionally, the difference in the fluorescent areas between ICG and vitamin $B_2$, as well as their luminance, was evaluated based on the video recording.

### Statistical analysis

All data were expressed as the mean ± standard deviation. The luminance of the fluorescent area and non-fluorescent area was compared using the Mann–Whitney U test, and the time-dependent change was compared using a one-way ANOVA. The StatMate IV software program (ATMS Co., Ltd., Tokyo, Japan) was used to perform the statistical analyses. $P$ values of $< 0.05$ were considered to indicate statistical significance.

## Results

### Experiment 1

Six healthy pigs were used (mean weight, 30.9 kg; range, 29.5–32.0 kg). In all animals, the fluorescent area and non-fluorescent area at the lung surface were clearly distinct. For at least 60 min, it was possible to detect yellow–green fluorescence in areas of the lung other than the cranial segment of the cranial lobe (Fig. 2a–d). Moreover, the line visualized with vitamin $B_2$ fluorescence matched the inflation–deflation line in all animals (Fig. 2e).

In the segmentectomy procedure for all six pigs, loose connective tissue thought to be the intersegmental plane was confirmed at the dissected surface; exposure or injury of the bronchus was not observed. The fluorescence remained in the lung until the intersegmental plane was dissected (Fig. 2f). After segmentectomy, the remaining cranial lobe caudal segment was sufficiently inflated in all animals. In the sealing test, the Macchiarini grade was 0 in four animals and 1 (minimal air leakage) in two animals. No obvious bronchial fistula was observed in either of the grade 1 animals.

The mean time required to visualize the intersegmental line after the intravenous injection of vitamin $B_2$ was 64 s (range 37–70 s). The time-dependent changes in luminance of the fluorescent and non-fluorescent areas are shown in Fig. 3. The luminance of the fluorescent area at 0, 5, 10, 20, 30, and 60 min after intravenous injection was 17.9 ± 3.5, 65.4 ± 11.0, 60.8 ± 5.8, 59.5 ± 5.7, 53.0 ± 5.1, and 51.5 ± 7.4, respectively. The luminance of the adjacent non-fluorescent area at 0, 5, 10, 20, 30, and 60 min after intravenous injection was 17.4 ± 3.9, 18.9 ± 3.0, 18.1 ± 2.9, 17.5 ± 2.4, 16.8 ± 0.7, and 17.0 ± 1.3, respectively. At

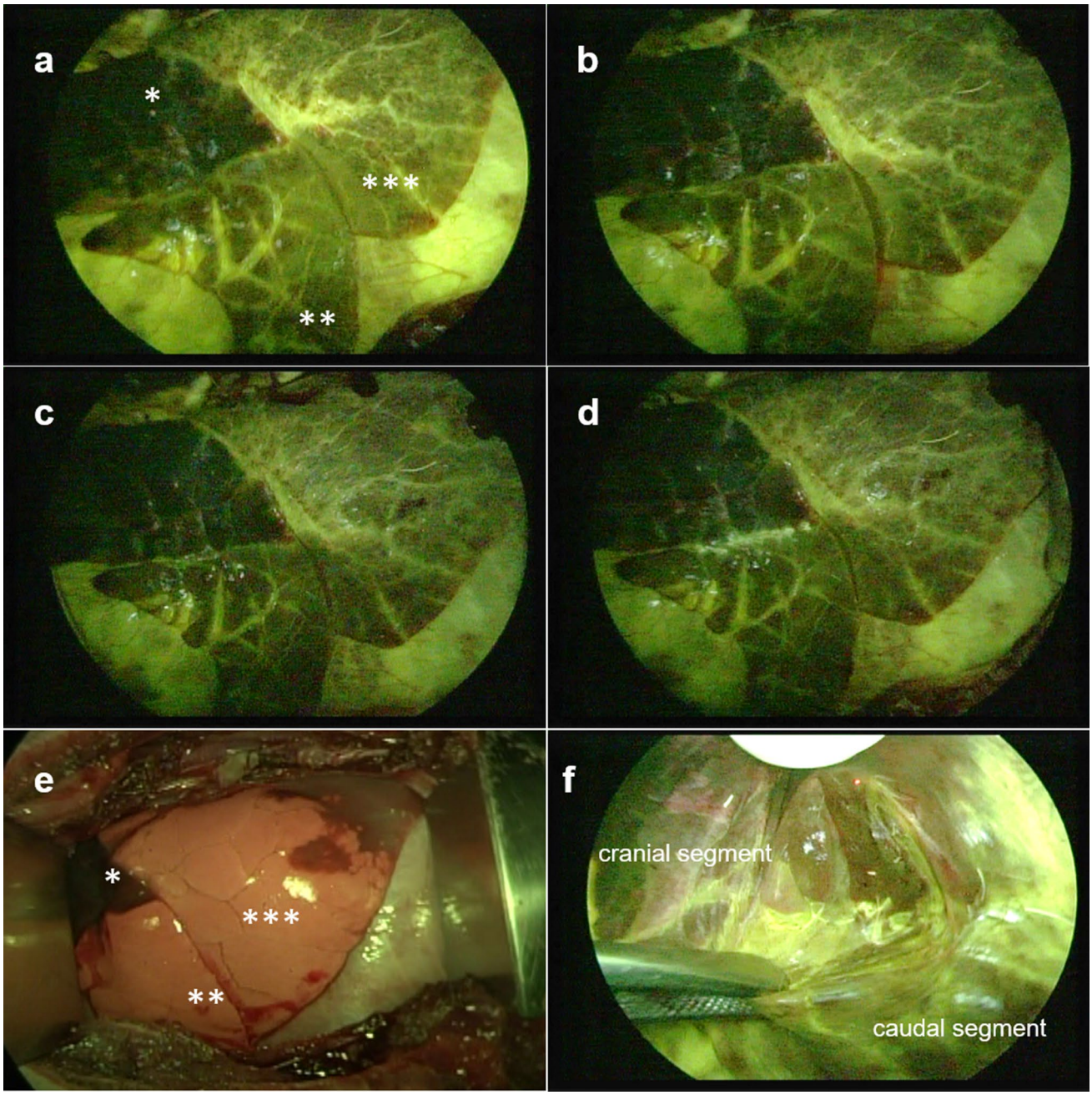


**Fig. 2** Observation of the intersegmental plane by a fluorescent camera after the intravenous injection of vitamin $B_2$. Fluorescence was observed at 5 min (**a**), 10 min (**b**), 20 min (**c**), and 30 min (**d**) after the intravenous injection of vitamin $B_2$. **e** White light observed during the creation of the inflation–deflation line. **f** Fluorescence observed inside the lung parenchyma during dissection of the lung parenchyma. *Cranial segment of the cranial lobe, **caudal segment of the cranial lobe, ***caudal lobe

5, 10, 20, and 30 min after intravenous injection, the luminance of the fluorescent area was significantly stronger than that of the adjacent non-fluorescent area ($p < 0.001$). The luminance of the fluorescent area gradually decreased over time, and a significant difference was observed between 5 and 30 min after intravenous injection ($p < 0.001$). However, the pulmonary intersegmental plane was still identified after 30 min, and the fluorescence at the unresected caudal segment of the cranial lobe continued for ≥ 60 min.

## Experiment 2

Six healthy pigs (mean weight, 31.8 kg; range 30.0–34.6 kg) were used for the comparison between vitamin $B_2$ and ICG. In all animals, the area of vitamin $B_2$ fluorescence

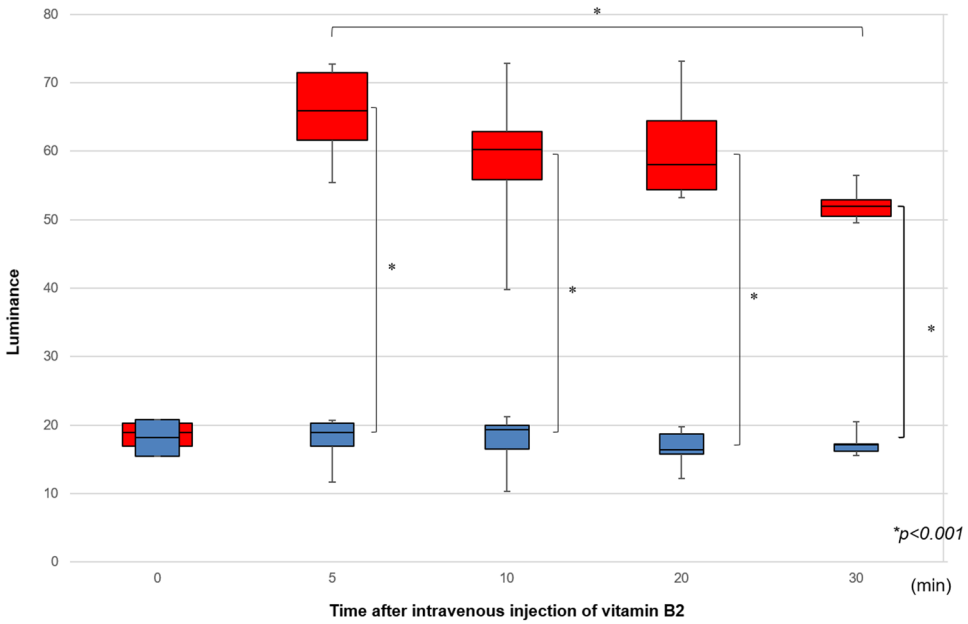


**Fig. 3** Comparison of fluorescence intensity between the fluorescent area and the non-fluorescent area after the intravenous injection of vitamin $B_2$. Red, fluorescent area; blue, non-fluorescent area

corresponded to the area of ICG fluorescence (Fig. 4). The area of ICG fluorescence was also clearly visualized, but its luminance was $44.2 \pm 3.2$, which was slightly less than that of vitamin $B_2$. The luminance of the non-fluorescent area was $23.8 \pm 1.2$.

## Discussion

In this study, the pulmonary intersegmental plane was identified in a pig model using intravenous vitamin $B_2$ and a fluorescent camera in all animals. The pulmonary intersegmental line visualized with the present method matched that of the inflation–deflation line. Moreover, the area of fluorescence was the same as that after the intravenous injection of ICG. Observation by the naked eye revealed that the degree of fluorescence after the intravenous injection vitamin $B_2$ was slightly weaker that after the intravenous injection of ICG; however, after the intravenous injection of vitamin $B_2$, the fluorescence continued for a longer time. Our method is useful in intersegmental dissection, not only for the lung surface, but also for the lung parenchyma.

Some early-stage, small-sized lung cancers can be completely cured with sublobar resection, and pulmonary segmentectomy is considered to be a useful radical procedure. Landreneau et al. [16] reported that in patients undergoing treatment for stage I small-sized lung cancer, the disease-free survival, overall survival, and recurrence rates of a segmentectomy group and a lobectomy group did not differ to a statistically significant extent. One advantage of segmentectomy over lobectomy is that it is possible to preserve the postoperative respiratory function [17, 18]. However,

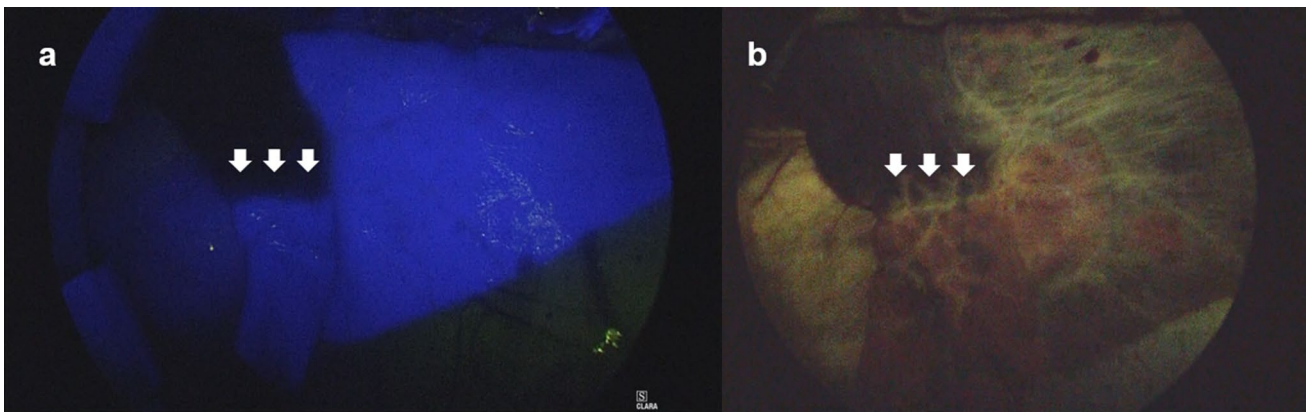


**Fig. 4** Areas of vitamin $B_2$ (**a**) and ICG (**b**) fluorescence. Arrows indicate the intersegmental plane

segmentectomy is a more complicated surgical procedure than lobectomy. Optimal segmentectomy requires accurate identification of the pulmonary intersegmental plane.

The pulmonary artery and bronchus generally follow a similar path [19], and the pulmonary vein is present at the pulmonary intersegmental plane. Thus, the pulmonary vein has classically been used as a guide for identifying the pulmonary intersegmental plane. However, the pulmonary vein varies greatly [20], and segmentectomy in which only the pulmonary vein is used as a guide is often associated with challenging procedures. Thus, there are currently several methods to visualize the pulmonary intersegmental lines at the pleura for use as a guide for the lung surface.

As a method to identify the pulmonary intersegmental lines on the lung surface, many institutions have used a technique that creates an inflation–deflation line by selectively opening or obstructing the bronchus of the target segment [4, 21]. However, it is difficult to determine the border in the lung parenchyma during intersegmental dissection. Moreover, air commonly flows through collateral ventilation, such as Kohn's pores and Lambert's canals, into an adjacent segment for which resection is not planned, especially in emphysematous lungs.

Recently, methods to identify the pulmonary intersegmental planes using the bronchial administration of fluorescent substances [5, 9, 10] (instead of air) have been reported. However, the infusion of air or an aqueous solution of a fluorescent substance into the bronchus inflates the lung. This can lead to problems with transection of the bronchus and vessels in the hilum and the removal of the resected lungs, because the operative field is narrow and the wound is small in surgeries performed under complete thoracoscopic surgery or with minimal access open surgery, which reduces the maneuverability of the surgical instruments. Moreover, an aqueous solution commonly flows through collateral ventilation into an adjacent segment, making the intersegmental plane ambiguous, as it is in methods to identify the intersegmental plane using a deflation-inflation line.

In pulmonary intersegmental plane identification, the use of the pulmonary artery blood flow, which is nearly identical to the bronchial path, may minimize the effects of emphysematous lungs. Recently, with the development of fluorescence-processing technology and thoracoscopy systems, the intravenous injection of ICG has come to be commonly and widely used in Japan, and it has often been reported [4, 7, 22–24]. According to Misaki et al., the mean duration required to attain visualization after the intravenous injection of ICG is 13 s, and the duration of fluorescence is short (3 min and 30 s) [25]. Although it is possible to only use intravenous ICG for marking the intersegmental line at the lung surface, the duration of fluorescence is very short to dissect the lung parenchyma, which makes it difficult to accurately identify the intersegmental plane. For this reason, the intersegmental plane must be dissected with a stapler after marking the pleura [26], resulting in negative effects on the preservation of the respiratory function, which is an advantage of segmentectomy [27]. In the present study, the fluorescent area after the intravenous administration of vitamin $B_2$ matched that after the intravenous administration of ICG in the same pig; however, the duration of fluorescence was longer in comparison to that after ICG. The duration of fluorescence after the intravenous administration of vitamin $B_2$ was $\geq$ 60 min. The pulmonary intersegmental plane could indeed be visualized during dissection of the lung parenchyma. We believe this could allow for sufficient time, even to perform fluorescence-guided dissection of the lung parenchyma.

The other advantage of the present method is the safety of vitamin $B_2$ as a fluorescent substance. Vitamin $B_2$ is water-soluble, does not induce hypervitaminosis, and rarely triggers allergic reactions [13]. Even with excess intake, the surplus of vitamin $B_2$ is rapidly eliminated in urine. The half-life is 1.1 h [28]. The mass intravenous or oral administration of vitamin $B_2$ was not reported to be associated with any side effects [28, 29]. In contrast, ICG has a short half-life of 3–4 min because it is selectively taken up by the liver after intravenous injection and is rapidly eliminated into the bile [30]. Thus, multiple intravenous injections of ICG may be necessary, potentially increasing the risk of complications. Moreover, serious side effects, such as shock and anaphylactoid symptoms have been reported [31]. The use of ICG is also contraindicated in patients with iodine allergy.

The present study was associated with some limitations. One limitation is that the optimal dose of vitamin $B_2$ has not been established. Although a vitamin $B_2$ dose of 200 mg/body was used in the present study, this would equate to approximately 400 mg/body in humans, when taking into account, the circulating blood volume. While 400 mg/body would not be a problematic dose [29], the determination of the optimal dose for clinical application in humans is a future task. The second limitation involves the restrictions of the endoscopic system for PDD that was used as the fluorescent camera. This system is not equipped with an appropriate wavelength range, for irradiating excitation light and observing fluorescence, to maximally extract the fluorescence from vitamin $B_2$. Thus, to improve the visualization of the fluorescent area, it is necessary to develop a dedicated filter for vitamin $B_2$. A third limitation is that this experiment used a young and healthy animal model rather than humans. It is, therefore, necessary to verify whether similar results would be obtained in patients with underlying pulmonary conditions.

In conclusion, the method of identifying pulmonary intersegmental planes with the intravenous injection of vitamin $B_2$ using a fluorescent camera could detect intersegmental planes in a pig model. Furthermore, it facilitated

intersegmental dissection not only at the lung surface, but also at the lung parenchyma. This method is simple, safe, and accurate, and is a useful alternative to the inflation–deflation technique and the intravenous ICG method. We are planning to conduct clinical trials.

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## Compliance with ethical standards

**Conflict of interest statement** The authors declare no conflicts of interest in association with the present study.

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