

# Design of a Surgical Pen-type Probe for Real-time Indocyanine Green Fluorescence Emission Diagnosis

**Ki-Cheol Yoon<sup>1,2</sup>**

<sup>1</sup>Dept. of Biomedical Engineering, College of Medicine, Gachon University  
38-13, Dokjom-ro 3, Namdong-gu, Incheon 21565, Republic of Korea

<sup>2</sup>Medical Devices R&D Center, Gachon University Gil Medical Center  
21, 774 beon-gil, Namdong-daero Namdong-gu, Incheon 21565, Republic of Korea  
e-mail kcyoon98@gachon.ac.kr

**Kwang Gi Kim<sup>1,2,3,4\*</sup>**

<sup>1</sup>Dept. of Biomedical Engineering, College of Medicine, Gachon University  
38-13, Dokjom-ro 3, Namdong-gu, Incheon 21565, Republic of Korea

<sup>2</sup>Medical Devices R&D Center, Gachon University Gil Medical Center  
21, 774 beon-gil, Namdong-daero Namdong-gu, Incheon 21565, Republic of Korea

<sup>3</sup>Dept. of Biomedical Engineering, College of Health Science, Gachon University  
191 Hambakmoero, Yeonsu-gu, Incheon 21936, Republic of Korea

<sup>4</sup>Dept. of Health Sciences and Technology, Gachon Advanced Institute for Health  
Sciences and Technology (GAIHST), Gachon University  
38-13, 3 Dokjom-ro, Namdong-gu, Incheon 21565, Republic of Korea  
e-mail kimkg@gachon.ac.kr

**Seung Hoon Lee<sup>5,6</sup>**

<sup>5</sup>School of Medicine, Eulji University  
77 Gyeryong-ro 771 Beon-gil, Jung-gu Daejeon 34824 Republic of Korea

<sup>6</sup>Dept. of Neurosurgery, Daejeon Eulji Medical Center, Eulji University  
95, Dunsanse-ro, Seo-gu, Daejeon 35233 Republic of Korea  
e-mail nslsh@eulji.ac.kr

## ABSTRACT

*The advantage of handheld type surgical microscope is that the size of the probe is small and light, and that both the working distance(15 to 30 cm) and field of view (30°) can be adjusted. The shortness working distance will minimize the loss of light source energy. However, the currently developed handheld type surgical microscope is still large, heavy, and uses relatively high energy (600 mW/cm<sup>2</sup>). To address the aforementioned problems, this study aimed to develop a pen-type surgical fluorescence microscope that is*

---

\*Corresponding Author : Kwang Gi Kim, e-mail : [kimkg@gachon.ac.kr](mailto:kimkg@gachon.ac.kr), Gachon University

*compact, portable, and has an adjustable beam angle and working distance. These features enable real-time diagnosis.*

*The pen-type probe consists of a laser diode, CMOS camera, light source brightness control device, filter, and power switch. The IR-cut filter inside the CMOS camera was removed to facilitate transmission of the fluorescence emission wavelength. In addition, a long-pass filter was attached to the camera so that the external light source was blocked and only the fluorescence emission wavelength was allowed to pass through. The performance of the pen-type probe was tested through a large animal experiment. Indocyanine green (2.5mg/kg) was injected into a pig's vein. Fluorescence emission of 805-830 nm was achieved by irradiating an external light source (785 nm and 4 mW/cm<sup>2</sup>), and liver-uptake occurred after 2.4 minutes. The designed pen-type probe was capable of sufficiently fluorescence expression through low-energy irradiation, and the pen-type probe is small and light and easy to handle by hand because both the pen-based laser device and the camera device are integrated. In addition, it is easy to adjust the working distance and field of view.*

## **INTRODUCTION**

Surgical diagnosis with indocyanine green (ICG) fluorescence dye is used to observe the flow conditions in blood vessels in the stomach, intestine, organ, liver, lymph node, and brain [1]-[5]. In particular, malignant tumors are complex and consist of tumors and blood vessels. Unfortunately, tumors and vasculature have the same color, making it difficult to identify the boundary between them with the naked eye. To observe the boundary between tumors and blood vessels, cancer surgery involves the use of surgical observation systems [2], such as surgical microscopes [6]-[11].

However, the disadvantages of such cameras are that they are large and heavy (camera head: above 4 kg, whole: above 6 kg) with limited portability. In addition, they

allow limited adjustments of the observed beam angles (maximum 30°) and working distances 15–30 cm) [11]-[21]. Because a surgical camera uses a high-energy light source (50–160 mW/cm<sup>2</sup>) as the electromotive force of fluorescence emission, optical source beam energy loss (above 58%) of the working distance (15–30 cm) and strong ambient light around the tissue (photobleaching) can occur [8], [11]. In other words, the surgical microscope is difficult to apply in tissue observation because of the limited beam working distance control and adjustment of the left and right of the camera [12], [15]. Moreover, surgical diagnostic systems are not portable because of their large size [8]. Several studies have been conducted on handheld surgical microscopes to overcome these problems [11], [22]. The advantage of a handheld surgical microscope is that its size and working distance are less than those of a conventional surgical microscope. In addition, a handheld microscope offers a wide range of photography angles. In surgical microscopes, Novadaq SPY Elite and Iridium vision sense and Karl storz vitom ICG have a smaller and lighter camera part equipped with a light source [19]. Nevertheless, Hamamatsu, Novadaq SPY PHI, Fluoptics fluobeam, and Quest medical spectrum are also smaller and lighter in handheld form. Therefore, sufficient handling became possible during the surgical procedure [19]. However, the feature of these is that the camera part integrated with the light source is small and light, so it is possible to operate the surgery in a handheld form, but the camera and the monitoring device are not integrated. That is, the camera and the monitoring device are separated from each other, and the monitoring device is quite large, which occupies a heavy and narrow surgical space for transport. Therefore, for this reason, the dimensions of handheld microscopes remain problematic. For example, although the

device introduced in [20] is light-weight, it is excessively long. Meanwhile, although the working distance of the device reported in [17] (which uses image-guided Raman spectroscopy) is short, it requires an optical light source capable of producing high energy ( $> 600 \text{ mW/cm}^2$  @ 405 nm) for excitation. In particular, light source generators and monitoring devices are configured in a single system, with cameras and lasers exposed through wires. The camera and laser are not integrated and are independently configured. Moreover, the construction of the system is complex, which causes difficulties in using the camera and light source separately and necessitates substantial manpower for the surgery.

To solve the problems of the surgical fluorescence microscope, this study proposes a pen-type probe that is small and portable for real-time diagnosis. The performance of the proposed pen-type probe was evaluated through animal experiments using a light source of 785 nm and a fluorescence wavelength of 810 nm. During the animal experiment, the pig's brain was incised for the pen-type probe performance test, and an ICG fluorescence contrast agent was injected into the blood vessels of the brain, and an external light source was irradiated to the blood vessels to observe the blood circulation state. The reason is that numerous blood vessels are distributed in brain tumors. After extraction of the tumor, an ICG fluorescent contrast agent is used to observe the blood flow state of the blood vessels. At this time, the performance was evaluated as to whether the proposed pen-type probe could sufficiently observe the blood flow state of the blood vessels distributed in brain tumors.

The characteristics of ICG fluorescence contrast diagnosis have been approved by the United States Food and Drug Administration [12, 13]. Therefore, it can be applied to observe the conditions of the heart, liver, blood flow, and blood vessels; and in angiography, cerebrovascular arteriovenous malformation, and vascular suture surgery [13], [23]-[25], [27].

## **DESIGN OF THE PEN-TYPE PROBE**

A proposed pen-type probe consists of a laser diode (LD), drive module (for laser drive), small camera module (SE-8J200), switch (ON/OFF), laser brightness control performance, battery, long pass filter, and communication cable as shown in Fig. 1. From the figure, the cable connects between pen-type probe and external monitor. The fluorescence emission imaging captured by the camera can be seen in real time on an external monitor through a cable.

The Thorlabs L785P090 laser diode was used, with excitation wavelength ( $\lambda_{\text{ext}}$ ) and maximum optical source power ( $P_{\text{ext}}$ ) of 785 nm and 19.1 mW, respectively, and operating voltage ( $V_{\text{in}}$ ) of 2.0 V. Because the laser diode generates thermal energy, thermal energy control is essential. Thus, the laser diode was connected to a photodetector (PD) that can detect and control the heat generated by the laser diode. When heat is generated, the control unit of the drive module adjusts to the heat after its detection by the photodetector.

The Thorlabs MLD203P2 drive module was used, with an operating voltage and current of DC 5 V and 200 mA, respectively. A variable resistor (max. 10 k $\Omega$ ) was used for

laser brightness control; therefore, the laser diode can adjust the optical source power. An endoscope camera was used in the complementary metal oxide semiconductor (CMOS) sensor. The camera has an operating voltage of 3 V. Its horizontal length and diameter are 4 mm and 1.5 mm.

The resolution, imaging frame, pixel size, field of view (FOV), and sensor type of the camera are  $1600 \times 1200$ , 30 fps,  $2.0 \text{ M}_{\text{pixel}}$ ,  $60^\circ$ , and CMOS sensor, respectively. The irradiation beam angle of the laser diode is free, i.e.,  $360^\circ$ . The working distance is 10 mm. Then, the working distance was adjusted from 0 to 50 cm, and the realization environment was measured as 50 mm.

The infrared (IR)-cut filter of an internal camera structure is removed. Fluorescence dye (ICG) imaging could not be achieved because the emission wavelength ( $E_m$ ) of ICG fluorescence dye imaging is in the IR band (above 800 nm) [4], [12, 13], [28]. Thus, the IR band ( $>800 \text{ nm}$ ) cannot transmit the IR-cut filter in the camera.

The camera lens (head) was connected to a long-pass filter with a cut-on wavelength of 800 nm. The long pass filter was fabricated using titanium oxide ( $\text{Ti}_3\text{O}_5$ ), silicon oxide ( $\text{SiO}_2$ ), and a glass-coated substrate, as shown in Fig. 2. The fabricated filter has a  $t$ ,  $l$ , and  $h$  of  $2 \text{ }\mu\text{m}$ ,  $4 \text{ }\mu\text{m}$ , and  $0.4 \text{ }\mu\text{m}$ , respectively; its diameter is 1.5 mm.

The long pass filter only transmits the wavelengths emitted by the ICG fluorescence dye and produces a diagnostic image without any excitation wavelength. The measurement results of the fabricated long-pass filter are shown in Fig. 3. The cut-in wavelength, transmission (T), and reflection (OD: optical density) are 805 nm, 98%, and 0.2%, respectively.

The pen-type probe was equipped with a low-cost camera. Fig. 4 shows the fabrication of the pen-type probe by 3D printing.

The pen-type probe is lighter than the conventional surgical microscope, with an overall size and weight of 17 mm × 2.5 mm and 32 g ( $\pm 2$  g), respectively; the conventional surgical microscope weighs 6.0 kg [14].

## EXPERIMENTAL RESULTS

A large animal (pig) experiment was performed to test the performance of the proposed pen-type probe. The animal was tested at the animal center of Osong Medical Innovation Foundation, KBIO (Osong Medical Innovation Foundation, KBIO). We obtained permission from the Animal Institutional Review Board (IRB) of the Animal Ethics Commission. In the animal test, the conventional-type pig (Farm / male (1ea) / weight of 60 kg). An ICG fluorescent contrast agent was injected into the pig vein.

The fluorescence expression of the agent was determined according to the concentration. The fluorescent contrast agent was mixed with a 10 ml aqueous injection solution and 25 mg of ICG (Dongindang, Republic of Korea, one vial 25 mL/insurance code: 664100231). The mixture (0.2–0.4 ml) was injected into the pig vein by a syringe. The injected ICG fluorescent material binds to plasma protein [29].

Fig. 5 shows the surgical environment for the animal test. As shown in the figure, the pen-type probe is connected to an external monitor by a cable.

The working distances were determined as 15 cm (conventional surgical microscope and NIR camera) and 50 mm (pen-type probe). However, the working distance of the pen-type probe is free between 50 mm and 50 cm.

For the animal test of ICG fluorescence emission, the optical source of the pen-type probe was irradiated to the vasculature of a pig. On the one hand, in order to prove the excellence of pen-type probe performance in animal experiments, we tested pen-type probe together with existing surgical microscopes. The conventional surgical microscope consists of an NIR camera and a laser (785 nm).

The excitation wavelength ( $\lambda_{\text{ext}}$ ) and laser power with the conventional surgical microscope and NIR camera (Lt 225 NIR M/N, respectively) were 785 nm and 60 mW/cm<sup>2</sup>, respectively. Then, the laser (785 nm) was used in the PerkinElmer Solaris optical imaging system and Thorlabs CPS780S (for NIR camera). The excitation wavelength ( $\lambda_{\text{ext}}$ ) and LD power of the pen-type probe were 785 nm and 4.0 mW/cm<sup>2</sup>, respectively.

The excitation wavelength ( $\lambda_{\text{ext}}$ ) and laser diode power of the pen-type probe were 785 nm and 4.0 mW/cm<sup>2</sup>, respectively. The laser diode (Thorlabs L785P90) power was adjusted from 0 mW/cm<sup>2</sup> to 19.0 mW/cm<sup>2</sup>. The camera used was the SE-8J200 (endoscopy-type microscopy). When the laser diode of the pen-type probe was irradiated with a wavelength of 785 nm (4.0 mW/cm<sup>2</sup>) to a blood vessel, fluorescence was emitted from the blood vessel and the blood flow state could be observed. The fluorescence emission wavelength and power ranged from 805 to 830 nm [4], [12, 13], [28].

Fig. 6 shows the resulting fluorescence emissions in vascular flow experiments on large animals, including the state of blood flow through ICG fluorescence in blood vessels.

Fluorescence emission images of blood flow were collected using an IR camera. The collected images could be observed through a cable.

Conventional surgical microscopes have poor clarity even though the light source irradiation power is 55-160 mW/cm<sup>2</sup> at a working distance of 15-30 cm. The reason is that when the working distance is 200 mm or more, the intensity of the light source decreases by 58% or more [24]. Therefore, fluorescence expression may be weak. In addition, it is filmed in black and white because of the characteristics of the NIR camera. However, the proposed pen-type probe minimizes the loss of light source intensity due to nearby working distance and allows color photographing on the camera, which can provide high resolution.

Fluorescent contrast agents are injected into the veins to observe blood circulation. In the figure, purple is the tissue around the blood vessel and white is showing the blood circulation state. When a fluorescent contrast agent is injected into the blood vessel and monitored externally through camera photography, it appears white in the blood vessel.

The reason why surrounding tissues look purple is because of the influence of the CMOS sensor of the camera. The first picture on the left in the experimental method is the step before injection of the fluorescent contrast agent. The experimental sequence turns off the lighting in the operating room when irradiating an external light source after injecting a fluorescent contrast agent. Therefore, darken the operating room to minimize external light noise. And after injecting a fluorescent contrast agent while irradiating an external light source, the blood circulation state of the blood vessels is observed on an external monitor.

The fluorescent contrast agent has a liver uptake phenomenon owing to its half-life of 3.4 min. Then, the [4], [28], [30] of ICG fluorescence has average 2.4 min (0.01 mg/kg) liver uptake phenomenon.

## DISCUSSION

A pen-type probe consists of a battery. The pen-type probe is connected via a camera, monitor, and USB cable. The camera will operate at a voltage of 3V but will receive a 5V supply from the monitor. Meanwhile, the drive module of the laser diode operates at 5V. At this point, the power to the drive module of the laser diode is connected to the power of the camera module and operates at 5V supplied by the monitor. However, the pen-type probe is equipped with a separate battery. The reason is that batteries can be used when installing a wireless camera in a pen-type probe and can be used as a spare power source when the power supply is unstable in the operating room.

Pen-type probes need to consider the importance of batteries because they need to test their performance using wireless cameras in the future to increase portability. The pen-type probe's camera appears to be better than the others, as shown in Table 1.

The resolution, frame, and pixel size of a pen-type probe camera is excellent (more than two times) and better than that obtained in previous studies [11], [17, 18]. However, Spy PHI has resolution and frame rate of 1080P and 60 fps, respectively [32]. Of course, the Spy PHI image imaging performance will be much better than the pen-type probe. However, the SPY PHI has a high unit price because the main body system equipped with monitoring is and depends on imports. Also, the maintenance cost is high as well as the unit price. In addition, Spy PHI has to be moved from the storage area to be used in the

operating room, and it is difficult to move it in a narrow operating room because of its heavy. In addition, the system takes up a lot of volume in a narrow operating. The external irradiation optical source power (laser module) of a conventional surgical microscope was 600–800 mW/cm<sup>2</sup>, and the working distance was 0.5 mm and 200–260 mm [11], [17, 18], [33]–[35]. However, the external irradiation optical intensity will have losses of approximately 58% when the working distance is above the minimum of 200 mm [24].

To generate ICG fluorescence emission, it is necessary to increase the optical source intensity or decrease the working distance. If the optical intensity of the laser diode (Thorlabs, L785PO) is increased, then the laser diode will cause thermal breakage because of its vulnerability to heat, as proven by the experiment. If the working distance of a conventional surgical microscope is decreased, then the field of view of the surgical lesion narrows [36].

The pen-type probe's laser diode enables the adjustment of the laser diode power and working distance. The pen-type probe's laser diode and working distance were adjusted to 4 mW/cm<sup>2</sup> and 50 mm, respectively, while the FOV was obtained as 60°. Thus, the vascular flow condition can be monitored according to ICG fluorescence emission. The maximum photographing angle of a conventional surgical microscope is 30–60° [37], while that of the pen-type probe is free (360°) [38]–[41]. Therefore, the pen-type probe does not have a blind spot, and the field of view of the surgical lesion is broader than that of the conventional surgical microscope. The camera of a pen-type probe is integrated the fundamentally CMOS sensor. The we have selected the CMOS sensor camera. The reason is as follows.

The size of a CMOS camera is smaller than the CCD camera due to simple structure of a module. The power consume of a CMOS sensor is lower than CCD camera, and the response time is significantly faster more than CCD camera. The FOV and dynamic range of a CMOS camera are wideness [42].

Laser irradiation should be conducted within a safe range of tissues, and in accordance with international medical standards (IEC 60601-2-41). The maximum irradiation power of a laser diode is 19 mW; the actual laser diode power used is within 4 mW; and is reduced by approximately 2.89 to 40 times. For this reason, it can be reduced to a small size, making it portable and suitable for a narrow operating room.

## **CONCLUSION**

In this paper, the design and fabrication for a pen-type probe is presented which has small size and it is possible to portable. The suggested pen-type probe is composed of a laser diode, an endoscope camera, a brightness control device of a light source, a power supply, a communication cable, and external monitor.

A pen-type probe can be observed the shape of tumor and blood vessel flow by difference of colors, and it can be observed the boundary between tumor and blood vessel through the difference of colors. In addition, a pen-type probe can observe the remanent state of a tumor by using fluorescence dye at an external monitor. In a pen-type probe, the detected image transfer to external monitor through the communication cable. A proposed pen-type probe has not an optical density loss nor a blind spot.

The pen-type probe is judged to be suitable for observing blood circulation after blood vessel or tumor extraction in the field of surgery or internal medicine in the future, and its application value is expected to be high.

#### **ACKNOWLEDGMENT**

The experiment was animal test from the “experimental animal center of Osong Medical Innovation Foundation, KBIO ((KBIO-IACUC-2019-021) through the permission of animal institutional review board (IRB) at animal ethics commission.

#### **FUNDING**

This research was financially supported by the Seoul R&D Program(BT190153) through the Research and Development for Regional Industry, and by the GRRRC program of Gyeonggi province. [GRRRC-Gachon2020(B01), AI-based Medical Image Analysis.

## NOMENCLATURE

|                 |                                         |
|-----------------|-----------------------------------------|
| ICG             | indocyanine green                       |
| LD              | <i>laser diode</i>                      |
| $\lambda_{ext}$ | excitation wavelength                   |
| $P_{ext}$       | optical source power                    |
| $V_{in}$        | operating voltage                       |
| PD              | photodetector                           |
| CMOS            | complementary metal oxide semiconductor |
| FOV             | field of view                           |
| IR              | infrared                                |
| $E_m$           | <i>emission wavelength</i>              |
| $Ti_3O_5$       | <i>titanium oxide</i>                   |
| $SiO_2$         | <i>silicon oxide</i>                    |
| $T$             | <i>transmission</i>                     |
| OD              | Reflection, optical density             |
| IRB             | Animal Institutional Review Board       |

## REFERENCES

- [1] Lefranc, F., Sadeghi, N., Camby, I., Metens, T., Dewitte, O., and Kiss, R., 2006, "Present and Potential Future Issues in Glioblastoma Treatment", *Expert Rev. Anticancer Therapy*, **6** (5), pp. 719-32. DOI: 10.1586/14737140.6.5.719
- [2] Stummer, W., Novotny, A., Stepp, H., Goetz, C., Bise, K., and Reulen, H. J., 2000, "Fluorescence-guided Resection of Glioblastoma Muliforme Utilizing 5-ALA-induced Porphyrins: A Prospective Study in Consecutive Patients", *J. Neurosurgery*, **93** (6), pp. 1003-13. DOI: 10.3171/jns.2000.93.6.1003
- [3] Pitskhelauri, D. I., Konovalov, A. N., Shekutev, G. A., Rojnin, N. B., Kachkov, I. A., Samborskiy, D. Y., Sanikidze, A. Z., and Kopachev, D. N., 2014, "A Novel Device for Hands-free Positioning and Adjustment of the Surgical Microscope", *J. Neurosurg*, **121** (1), pp. 161-164. DOI: 10.3171/2014.3.JNS12578
- [4] Dupr  ea, A., Rie  a, H., Detter, C., Debus, E. S., and Wipper, S. H., 2018, "Utilization of Indocyanine Green Fluorescent Imaging (ICG-FI) for the Assessment of Microperfusion in Vascular Medicine", *Innov. Surg. Sci.*, **3** (3), pp. 1193-201. DOI: 10.1515/iss-2018-0014
- [5] DSouza, A. V., Lin, H., Henderson, E. R., Samkoe, K. S., and Poguea, B. W., 2016, "Review of Fluorescence Guided Surgery Systems: Identification of Key Performance Capabilities Beyond Indocyanine Green Imaging", *J. of Biomedical Optics*, **21** (8), pp. 080901. DOI: 10.1117/1.JBO.21.8.080901
- [6] Unno, N., Suzuki, M., Yamamoto, N., Inuzuka, K., Sagara, D., Nishiyama, M., Tanaka, H., and Konno, H., 2008, "Indocyanine Green Fluorescence Angiography for Intraoperative Assessment of Blood Flow: A Feasibility Study", *European J. Vasc. Endovasc. Surg.*, **35** (2), pp. 205-207. DOI: doi.org/10.1016/j.ejvs.2007.09.001
- [7] Desmettre, T., Devoisselle, J. M., and Mordon, S., 2000, "Fluorescence Properties and Metabolic Features of Indocyanine Green (ICG) as Related to Angiography", *Elsevier Sci. Inc.*, **45** (1), pp. 15-27. DOI: 10.1016/s0039-6257(00)00123-5
- [8] Qi, B., Crawford, A. J., Wojtynek, N. E., Holmes, M. B., Soucek, J. J., Almeida-Porada, G., Ly, Q. P., Cohen, S. M., Hollingsworth, M. A., and Mohs, A. M., 2018, "Indocyanine Green Loaded Hyaluronan-derived Nanoparticles for Fluorescence-enhanced Surgical Imaging of Pancreatic Cancer", *Nanomedicine: Nanotechnology, Biology, and Medicine*, **14** (3), pp. 769-780. DOI: 10.1016/j.nano.2017.12.015
- [9] Son, G. M., Kwon, M. S., Kim, Y., Kim, J., Kim, S. H., and Lee, J. W., 2019, "Quantitative Analysis of Colon Perfusion Pattern Using Indocyanine Green (ICG) Angiography in Laparoscopic Colorectal Surgery", *Surgical Endoscopy*, **33** (5), pp. 1640–1649. DOI: 10.1007/s00464-018-6439-y

- [10] Delong, J. C., Ward, E. P., Lwin, T. M., Brumund, K. T., Kelly, K. J., Horgan, S., and Bouvet, M., 2018, "Indocyanine Green Fluorescence-guided Parathyroidectomy for Primary Hyperparathyroidism", *Surgery*, **163** (2), pp. 388–392. DOI: 10.1016/j.surg.2017.08.018
- [11] Hong, N. Y., H. Kim, R. H., Lee, M., Sohn, D. K., and Kim, K. G., 2016, "Fluorescent Property of Indocyanine Green (ICG) Rubber Ring using LED and Laser Light Sources", *Biomedical Optics Express*, **7** (5), pp. 1637-1644. DOI: 10.1364/BOE.7.001637
- [12] Alander, J. T., Kaartinen, I., Laakso, A., Patila, T., Spillmann, T., Tuchin, V. V., Venermo, M., and Välisuo, P., 2012, "A Review of Indocyanine Green Fluorescent Imaging in Surgery", *Hindawi Int. J. Biomedical Imaging*, **2012** (2012), pp. 1-26. DOI: 10.1155/2012/940585
- [13] Dsouza, A. V., Lin, H., Henderson, E. R., Samkoe, K. S., and Pogue, B. W., 2016, "Review of Fluorescence Guided Surgery Systems: Identification of Key Performance Capabilities Beyond Indocyanine Green Imaging", *J. of Biomedical Optics*, **21** (8), pp. 08090(1) – 08090(15). DOI: 10.1117/1.JBO.21.8.080901
- [14] Watson, J. R., Gainer, C. F., Martirosyan, N., Skoch, J., Michael Lemole, G., Anton, R., and Romanowski, M., 2015, "Augmented Microscopy: Real-time Overlay of Bright-field and Near-infrared Fluorescence Images", *J. of Biomedical Optics*, **20** (10), pp. 106002. DOI: 10.1117/1.JBO.20.10.106002
- [15] Wen, C. T., Liu, Y. Y., Fang, H. Y., Hsieh, M. J., and Chao, Y. K., 2018, "Image-guided Video-assisted Thoracoscopic Small Lung Tumor Resection using Near-infrared Marking", *Springer Surg. Endosc.*, **32** (11), pp. 4673-4680. DOI: 10.1007/s00464-018-6252-7
- [16] Rabinovitz, H. S., Oliviero, M., and Scope, A., 2016, "Application of handheld confocal microscopy for skin cancer diagnosis advantages and limitations compared with the wide-probe confocal", *Dermatol Clin*, **34** (4), pp. 469–475. DOI: 10.1016/j.det.2016.05.009
- [17] Horgan, C. C., Bergholt, M. S., Thin, M. Z., Nagelkerke, A., Kennedy, R., Kalber, T. L., Stuckey, D. J., and Stevens, M. M., 2021, "Image-guided raman spectroscopy probe-tracking for tumor margin delineation", *Journal of Biomedical Optics*, **26** (3), pp. 036002 (1)-036002 (15). DOI: 10.1117/1.JBO.26.3.036002
- [18] Sajedi, S., Sabet, H., and Choi, H. S., 2019, "Intraoperative biophotonic imaging systems for image-guided interventions", *Nanophotonics*, **8** (1), pp. 99-116. DOI: 10.1515/nanoph-2018-0134

- [19] Dayani, P. N., Maldonado, R., Farsiu, S., and Toth, C. A., 2009, "Intraoperative use of handheld spectral domain optical coherence tomography imaging in macular surgery", *Retina*, **29** (10), pp. 1457-1468. DOI: 10.1097/IAE.0b013e3181b266bc
- [20] Kim, D., Hwang, K., Ahn, J., Seo, Y., Kim, J., Lee, S., Yoon, J., Kong, E., Jeong, Y., Jon, S., Kim, P., and Jeong, K., 2019, "Lissajous scanning two-photon endomicroscope for In vivo tissue imaging", *Scientific Reports*, **9**, pp. 1-8. DOI: 10.1038/s41598-019-38762-w
- [21] Hiroshima, Y., Maawy, A., Sato, S., Murakami, T., Uehara, F., Miwa, S., Yano, S., Momiyama, M., Chishima, T., Tanaka, K., Bouvet, M., Endo, I., and Hoffman, R. M., 2014, "Hand-held high-resolution fluorescence imaging system for fluorescence-guided surgery of patient and cell-line pancreatic tumors growing orthotopically in nude mice", *Journal of Surgical Research*, **187** (2), pp. 510-517. DOI: 10.1016/j.jss.2013.11.1083
- [22] <https://www.amazon.co.uk/Smallest-Handheld-Fluorescence-Microscope-Professional/dp/B00DD8W28O>.
- [23] Ma, L. and Li, B., 2021, "Comprehensive Review of Surgical Microscopes: Technology Development and Medical Applications", *J. of Biomedical Optics*, **26** (1), pp. 010901 (1). DOI: 10.1117/1.JBO.26.1.010901
- [24] Hoerenz, P., 1980, "The Operating Microscope I. Optical Principles Illumination Systems, and Support Systems". *J. of Microsurgery*, **1** (5), pp. 364-369. DOI: 10.1002/micr.1920010506
- [25] Klein, G., Baumgartner, R., and Flower, R., 1990, "An Image Processing Approach to Characterizing Choroidal Blood Flow", *Investigative Ophthalmology & Visual Science*, **31** (4), pp. 629-637. PMID: 2335432
- [26] Themelis, G., Yoo, J. S., Soh, K. S., Schulz, R., and Ntziachristos, V., 2009, "Real-time Intraoperative Fluorescence Imaging System Using Light-Absorption Correction", *J. Biomedical Optics*, **14** (6), pp. 064012-064012-064019. DOI: 10.1117/1.3259362
- [27] Hauser, Rück. C., Lorenz, S., Mosch, S., Rotte, S., Kessler, M., and Kalinina, S., 2013, "Cell Metabolism, Tumour Diagnosis and Multispectral FLIM. In SPIE BiOS", *Int. Society for Optics and Photonics, San Francisco, California, USA*, 85880U-85880U-85888. DOI: doi.org/10.1117/12.2003729
- [28] Cherrick, GR., Stein, SW., Leevy, CM., and Davidson, CS., 1960, "Indocyanine Green: Observation on Its Physical Properties, Plasma Decay and Hepatic Extraction", *J. of Clin Invest.*, **39** (4), pp. 592-600. DOI: 10.1172/JCI104072
- [29] Miwa, M., 2010, "The Principle of ICG Fluorescence Method", *The Open surgical Oncology Journal*, **2**, pp. 26-28. DOI: 10.2174/1876504101002010026

- [30] Hope-Ross, M., Yannuzzi, LA., Gragoudas, E. S., Guyer, D. R., Slakter, J. S., and Sorenson, J. A., 1994, "Adverse Reactions due to Indocyanine Green", *Ophthalmology*, **101** (3), pp. 529–533. DOI: 10.1016/s0161-6420(94)31303-0
- [31] Hirche, C., Engel, H., Kolio, L., Cognie, J., Hünerbein, M., Lehnhardt, M., and Kremer, T., 2012, "An experimental study to evaluate the fluobeam 800 imaging system for fluorescence-guided lymphatic imaging and sentinel node biopsy", *Surgical Innovation*, **20** (5), pp. 516–523. DOI: 10.1177/1553350612468962
- [32] <https://www.stryker.com/se/en/endoscopy/products/spy-phi/index-eu.html>
- [33] Eker, C., Montan, S., Jaramillo, E., Koizumi, K., Rubio, C., Andersson-Engels, S. Svanberg, K., Svanberg, S., and Slezak, P., 1999, "Clinical spectral characterisation of colonic mucosal lesions using autofluorescence and  $\delta$  aminolevulinic acid sensitization", *Gut*, **44** (4), pp. 511–518. DOI: 10.1136/gut.44.4.511
- [34] Georges, J. F., Valeri, A., Wang, H., Brooking, A., Kakareka, M., Cho, S. S., Al-Atrache, Z., Bamimore, M., Osman, H., Ifrach, J., Yu, S., Li, C., Appelt, D., Lee, J. Y. K., Nakaji, P., Brill, K., and Yocom, S., 2019, "Delta-aminolevulinic acid-mediated photodiagnoses in surgical oncology: a historical review of clinical trials", *Front. Surg.*, **04**, pp. 1-16. DOI: 10.3389/fsurg.2019.00045
- [35] Yoon, K. C., Kim, EJ., Kim, KG., and Lee, SH., 2019, "A Multi-detection Microscope with 5-ALA and ICG Using Dual Array LED", *Current Optics Photonics*, **3** (3), pp. 256-262. DOI: <https://doi.org/10.3807/COPP.2019.3.3.256>
- [36] Hoerenz, P., 1980, "The Operating Microscope II. Individual Parts, Handling, Assembling, Focusing, and Balancing", *J. of Microsurgery*, **1** (6), pp. 419-427. DOI: 10.1002/micr.1920010603
- [37] Lee, J., Wijesinghe, Jeon, R. E., D., Kim, P., Choung, Y. H., Jang, J. H., Jeon, M., and Kim, J., 2018, "Clinical Utility of Intraoperative Tympanomastoidectomy Assessment Using a Surgical Microscope Integrated with an Optical Coherence Tomography", *Scientific Reports*, **8** (1), pp. 1-8. DOI: 10.1038/s41598-018-35563-5
- [38] Ricci, F., Missiroli, F., and Cerulli, L., 2003, "Indocyanine Green Dye Enhanced Micro Pulsed Diode Laser: A Novel Approach to Subthreshold RPE Treatment in a Case of Central Serous Chorioretinopathy", *European J. Ophthalmology*, **14** (1), pp. 74-82. DOI: 10.1177/112067210401400115
- [39] Marshall, M. V., Rasmussen, J. C., Tan, I. C., Aldrich, M. B., Adams, K. E., Wang, X., Fife, C. E., Maus, E. A., Smith, L. A., and Sevick-Muraca, E. M., 2010, "Near-Infrared Fluorescence Imaging in Humans with Indocyanine Green: A Review and Update", *Open*

Surgical Oncology Journal (Online), **2** (2), pp. 12-25. DOI:  
10.2174/1876504101002010012

[40] Themelis, G., Yoo, J. S., and Ntziachristos, V., 2008, "Multispectral Imaging Using Multiple-bandpass Filters", Optics Lett., **33** (9), pp. 1023-1025. DOI:  
doi.org/10.1364/OL.33.001023

[41] Kondo, Y., Murayama, Y., Konishi, H., Morimura, R., Komatsu, S., Shiozaki, A., Kuriu, Y., Ikoma, H., Kubota, T., and Nakanishi, M., 2014, "Fluorescent Detection of Peritoneal Metastasis in Human Colorectal Cancer Using 5-Aminolevulinic acid", Int. J. Oncology, **45** (1), pp. 41-46. DOI: 10.3892/ijo.2014.2417

[42] <https://www.techbriefs.com/component/content/article/tb/supplements/ptb/features/articles/20063>

### Figure Captions List

- Fig. 1            Structure of a pen-type probe
- Fig. 2            Layout and structure of an optical long-pass filter
- Fig. 3            Experimental result of fabricated a long-pass filter
- Fig. 4            Fabrication of a pen-type probe using 3D printing technique
- Fig. 5            Conventional pig (Farm) surgery and large animal test (a) test using  
conventional surgical microscope and NIR camera with laser (b) pen-type  
probe test
- Fig. 6            Experimental results of large animal (a) conventional surgical microscope  
(b) pen-type probe camera

**Table Caption List**

Table 1      Comparison for performance of hand-held type surgical microscope

## Information Regarding Figures

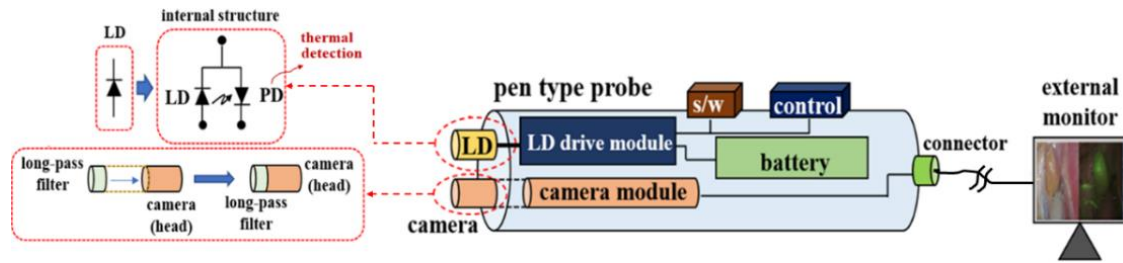


Fig. 1. structure of a pen-type probe.

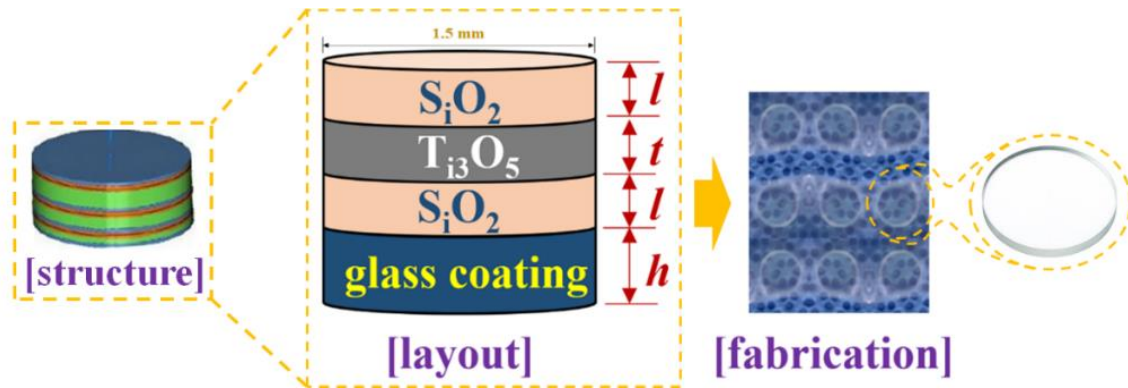


Fig. 2. Layout and structure of an optical long-pass filter.

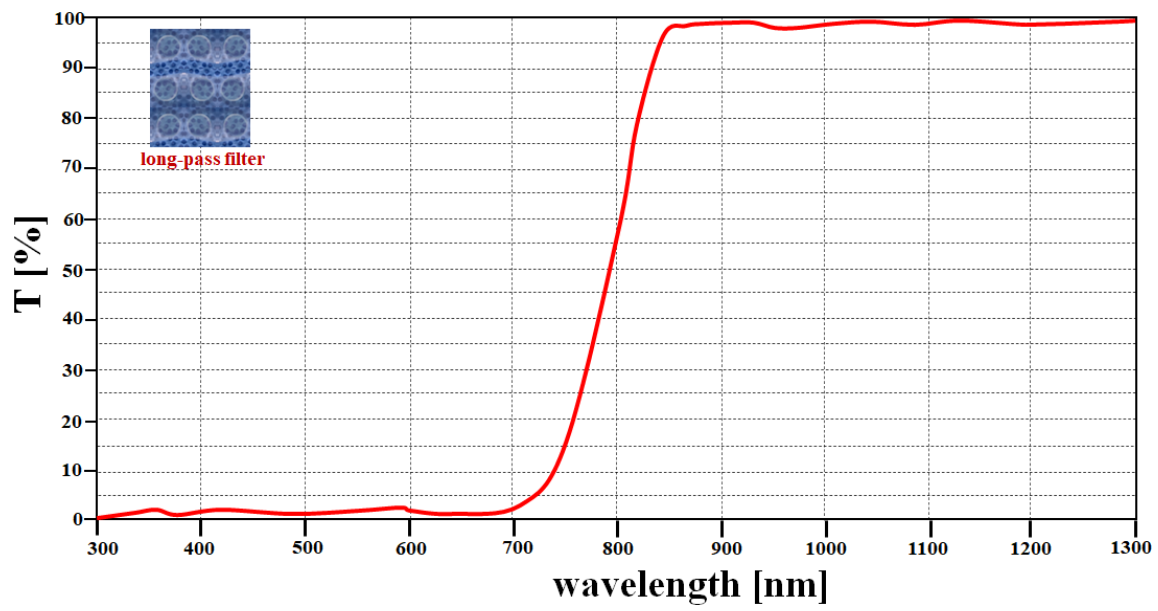


Fig. 3. Experimental result of fabricated a long-pass filter.

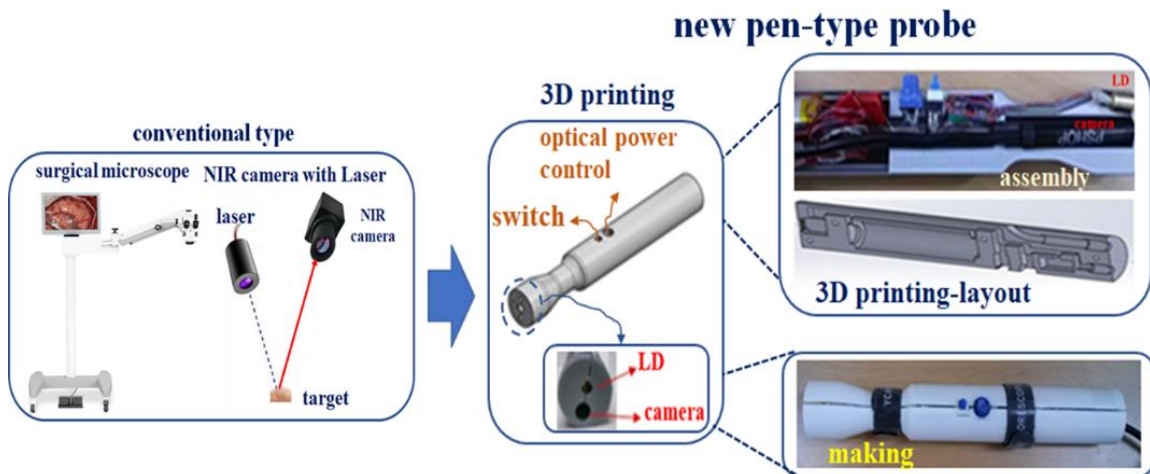


Fig. 4. Fabrication of a pen-type probe using 3D printing technique.

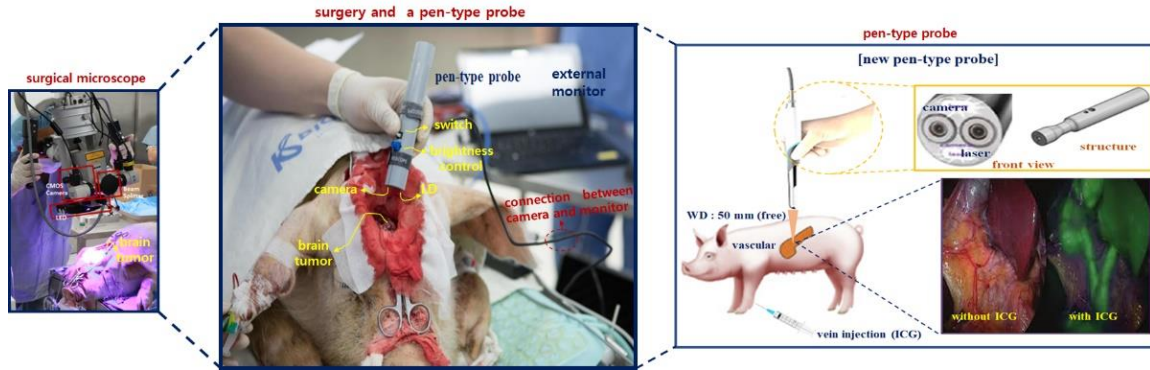


Fig. 5. Conventional pig (Farm) surgery and large animal test (a) test using conventional surgical microscope and NIR camera with laser (b) pen-type probe test.

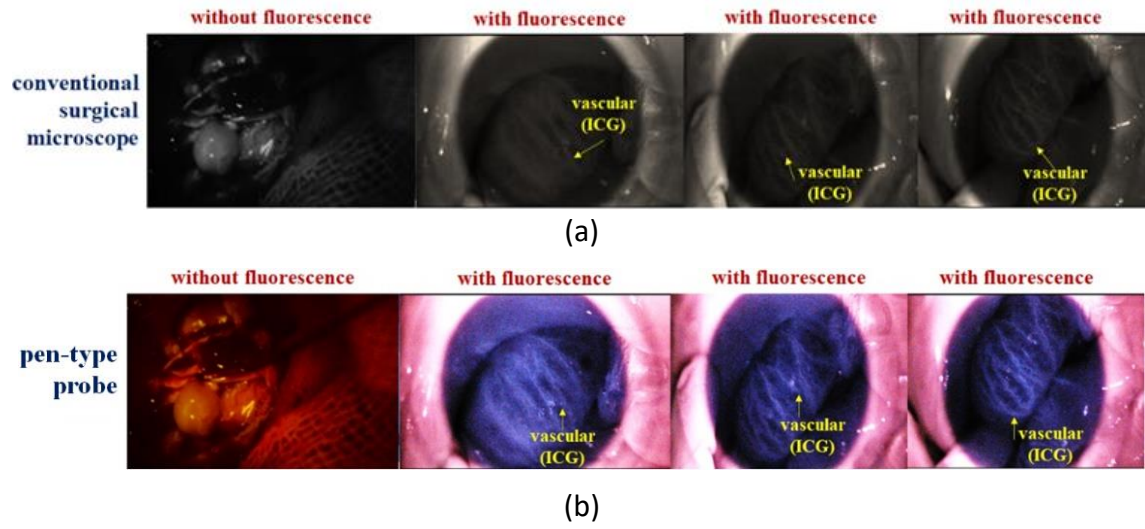


Fig. 6. Experimental results of large animal (a) conventional surgical microscope (b) pen-type probe camera.

### Information Regarding Tables

Table 1. Comparison for performance of hand-held type surgical microscope

|                                               | present<br>work | Raman<br>spectroscopy<br>probe [17] | fluobeam<br>[18], [33] | Spy PHI<br>[18], [33], [34] |
|-----------------------------------------------|-----------------|-------------------------------------|------------------------|-----------------------------|
| optical source<br>power [mW/cm <sup>2</sup> ] | 4.0             | 600                                 | 382                    | 800                         |
| working<br>distance [mm]                      | 50.0            | 0.50                                | 260                    | 30                          |
| resolution<br>[pixels]                        | 1600×1200       | 640×480                             | 720×576                | 1920×1080<br>(1080P)        |
| frame rate [fps]                              | 30              | 30                                  | 25                     | 60                          |
| FOV [mm <sup>2</sup> ]                        | 76×42           | 1.07×1.07                           | 20.0×14.0              | 19.0×13.0                   |
| sensor                                        | CMOS            | CCD                                 | CCD                    | CCD                         |