Title: A handmade, low-cost intraoperative fluorescence navigation system with indocyanine green for sentinel lymph node biopsy in skin cancer.

Keywords: indocyanine green, sentinel lymph node biopsy, fluorescence, intraoperative, skin cancer

Manuscript number of words: 2272(excl. abstract and references), 5 figures, and 2 tables

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Funding source: This work was supported in part by a Grant-in-Aid for Cancer Research(21S-7⑥) from the Japanese Ministry of Health, Labour, and Welfare.

Conflict of interest: We have no conflict of interest to report.

Abstract

Background: Recently, indocyanine green (ICG) fluorescence imaging has been reported as new method to detect sentinel lymph nodes (SLNs). However, high introduction costs limit its use.

Objective: The purpose of this study was to construct an ICG fluorescence imaging system by using parts commonly available on the market, thereby reducing costs.

Methods: We constructed this system using a charge-coupled device camera and light-emitting diodes. SLN biopsy using this system was performed in 16 patients with skin cancer.

Results: We could construct our system at a cost of less than $1,600. This system could observe lymphatic channels through the skin and detect SLNs. However, SLNs in the neck were difficult to detect through the skin.

Conclusion: Our system could be assembled at a reasonable cost and allowed us to detect SLNs efficiently. It may be used as an alternative to radiotracer for detecting SLNs located in the groin and axillary regions.

Introduction

The concept of the sentinel lymph node (SLN) biopsy was first reported by Morton et al[1]. This method can avoid unnecessary lymphadenectomy for melanoma patients with negative SLN metastasis. Since that report, SLN biopsy has been widely used in the management of a variety of cancers, such as gastric cancer [2] and breast cancer [3].

SLN biopsy has been performed mainly using two different techniques: injection of blue dye (dye method) and injection of radioisotope colloid (RI method). Using blue dye is safe, convenient, and cost-effectiv, but the technique has certain limitations, such as a loss of visibility in dense fat and rapid transit of the dye[4], [5]. Thus, combined use of the dye and RI methods provides high rates of SLN detection: rates of over 90 % have been reported [6], [7]. However, some surgeons have voiced concern about the adverse effects of using radioactive agents both for the medical staff and the patient [8]. Moreover, handling of RI is strictly regulated and the intraoperative hand-held gamma probe system for SLN mapping is very expensive, costing approximately $20,000 [9]. Thus, legal, safety, and cost considerations limit the use of the RI method in general hospitals.

Recently, Kitai et al. [10] reported on the SLN biopsy technique using indocyanine green (ICG) fluorescence imaging in breast cancer patients. Unlike the blue dye, this

ICG fluorescence penetrates human tissue to a depth of 1 to 2 cm and can detect the fluorescence transcutaneously in real time [11]. Although this ICG method would be of great benefit for hospitals where use of RI is not permitted, the ICG fluorescence detection system is very expensive, e.g., over $50,000 in Japan. Moreover, the surgery lamp should be turned off when detecting fluorescence because halogen lamps emit infrared light and ICG specific fluorescence cannot be detected. Repeatedly switching the lamp on and off leads to prolonged operation time.

In this study, we constructed an ICG fluorescence detection system (ICG-FDS) by using parts commonly available on the market to reduce costs and we compared the SLN detection rate with that of the conventional combined dye and RI method.

Materials and Methods

Instruments

The excitation wavelength of ICG that produces maximum fluorescence is 765 nm, and the fluorescence occurs at 840 nm in plasma [12]. Accordingly, we used a light-emitting diode (LED) that generates 760-nm wavelength light as the excitation light, and a charge-coupled device (CCD) camera with a long-wavelength pass filter to filter out light with a wavelength below 840 nm as the detector. All the parts used in this

ICG-FDS are listed in Table 1 For the construction of the ICD-FDS, we aligned 45 LEDs on a 75-mm diameter polyvinyl chloride (PVC) cap (Fig-1A). The CCD camera was set in the center of the cap. The main body consisted of a 75-mm diameter PVC pipe in the center of which we fixed the CCD camera (Fig-1B). The fluorescence image was sent to a TV monitor for real-time monitoring via a video recorder. When in use, the main body of the ICG-FDS was covered with a sterilized vinyl bag and the accessory cord, by a vinyl umbrella cover. We built this ICG-FDS at a cost of less than $1,600.

To gain sufficient visible light intensity without altering the detecting fluorescence, we mounted a short-wavelength pass filter which passes below 810 nm onto a common headlight (Fig-1C)

Before using this ICG-FDS in patients, we checked its performance by injecting ICG into the main author's forearm (Fig-2A). Immediately after the injection, lymph vessels were detected as several lines projecting toward the axillary basin (Fig-2B and C). Finally, we could trace the lymph flow all the way to an axillary lymph node (Fig-2D).

Procedure

Before the operation, lymphoscintigraphy was performed. On the morning of the operation, 0.4 to 0.6 mL (4-6 MBq) of 99Tc- tin colloid (Japan Mediphisics, Tokyo, Japan) was intradermally injected around the primary tumor. The interval between injection of

the tracer and earliest identification of a SLN varied from 5 to 20 minutes. Static images were taken and the skin overlying the detected SLN was marked before the patient was moved to the operation room. After the induction of general anesthesia, 0.3 to 0.6 mL of 2% patent blue solution and 0.5% ICG (Diagnogreen ;, Dai-ichi Pharmaceutical, Tokyo, Japan) were intradermally injected around the primary tumor and ultrasonography was then performed to localize potential lymph nodes. Wearing the headlight mounted with the short-wavelength pass filter on his head, the operating surgeon manipulated the ICG-FDS to track the fluorescence (Fig-1C). It should be noted that the operation site was well lit (Fig-1D) Basically, the skin incision line was determined according to the result of the lymphoscintigraphy. For operations in which the RI method was not used, the incision line was determined according to the result of the ICG-FDS with ultrasonography support. An intraoperative gamma probe system (Navigator GPS System; RMD Instruments,Watertown, MA, USA) was also applied to detect the SLN.

Patients

Sixteen patients with skin cancer were examined from December 2009 to July 2010 at the Department of Dermatology of the University of Tsukuba Hospital. Of these, 6 had melanoma, 5 had extramammary Paget' disease, 3 had squamous cell carcinoma, and 2

had cutaneous adenocarcinoma. Informed consent was obtained from every patient.

Results

The results are shown in detail in Table 2. The SLNs were successfully identified in all 16 patients, with 26 lymph node basins. The SLNs identified using the ICG-FDS were consistent with the results obtained by using the conventional methods: 95% using the RI method (19 in 20 lymph node basins) and 92% using the blue dye method (22 in 24 lymph node basins). On average, the number of SLNs was 1.5 per basin on average (1-3). In 6 patients, the SLNs were in 2 lymph node basins; in 1 patient, they were in the axillae and groin; and in the other patients, they were in the bilateral groin. Four patients had metastatic SLNs and received lymph node dissection.

In all cases, this ICG-FDS could detect the subcutaneous lymphatic stream (Fig-3A, B) flowing toward the lymph node basins and reached the SLN within 10 minutes. In 10 patients, the lymph flow draining into the SLN could be detected through the skin with the ICG-FDS (Fig-3C, D). In 6 patients, the fluorescence could not be traced to the SLN owing to its deeper location; however, in 2 of these cases, we could improve the fluorescence observation by pushing or pulling the peripheral skin and reducing the depth of fluorescence (Fig 4A and B). This improvement could not be achieved in the

other 4 because of the particular locations of the SLNs; under the primary tumor in 1 case, in a parotid gland and under the sternocleidomastoid muscle in 1 case, under the platysma muscle in 1 case, and in the axillae in the other case. However, in these 4 cases, the lymph channels could be observed after the skin incision, and the fluorescence to the SLN traced.

In 3 cases, the results of the ICG method were inconsistent with those of conventional methods (underlined part in Table 2). In cases 2 and 14, the ICG and RI method identified the SLNs in the bilateral groin and submandibular region, respectively but failed to show accumulation of the blue dye. In case 7, lymphoscintigraphy identified 1 SLN in the left groin, but the ICG method showed lymph flow toward the bilateral groin region (Fig. 4C and D). Both SLNs showed not only ICG but also blue dye accumulation. The reason for these discrepancies is uncertain, but we speculated that it might due to the difference of the injection sites, shorter interval between injection or lack of sufficient color intensity to permit identification.

A spotlight with a pass filter provided sufficient light intensity for the SLN biopsy procedure without altering the fluorescence detection. This lighting reduced the operating time because the conventional ICG fluorescence method requires shutting down the surgical light each time fluorescence is being detected.

Discussion

The concept of SLN biopsy is widely accepted in the management of various cancers, including breast, gastric, and skin cancers. However, this concept is based on accurate detection of the SLN, and false-negative SLN biopsy may lead to inappropriate assessment of the disease progression. The significance of the technical learning curve for SLN biopsy has been reported and substantial experience is required to develop the technical skill necessary to achieve a high success rate [4], [5]. The use of a radiopharmaceutical agent in addition to blue dye increased the success rate to 98% [4], but the use of radioisotopes is limited to large institutes and hospitals. Thus, a convenient, radioisotope-free, but reliable detection method is in great demand.

ICG is a popular diagnostic reagent used in a variety of examinations, including hepatic function, cardiac output, and retinal angiography [10]. It binds with albumin to produce a peak wavelength of 840 nm near-infrared fluorescence when excitated with 765 nm light [12]. Thus, ICG can provide an ideal lymphatic tracer for SLN mapping because it shows high intensity and contrast compared with blue dye [13] and its near-infrared fluorescence penetrates human soft tissue and allows detection to a depth of 1 to 2 cm under the skin. We can easily observe the lymph flow in real time and the draining SLN.

This real-time observation and visualization gives ICG its advantage over the handheld gamma probe method. Recently, several successful SLN biopsies using ICG fluorescence have been reported in breast cancer [10], [14] , gastric cancer [3], rectal cancer [15], and skin cancer [16].

Since medical insurance in Japan does not cover the extra fee of SLN biopsy for skin malignancies other than melanoma, it is difficult for hospitals to purchase expensive medical electronic devices for SLN biopsy. Use of the handheld gamma probe system is gradually becoming common in various institutions because the combined use of the RI and blue dye methods is considered the standard method of SLN biopsy. However, few institutes have the extra budget needed to purchase an ICG detection system in addition to the RI method. The principle of ICG fluorescence imaging is very simple and we thought that we could construct an ICG detection system by using parts commonly available on the market. As a result, we could build a system with sufficient ability to detect fluorescence in the clinical setting and at a reasonable cost.

On the other hand, the ICG fluorescence method has several limitations: (1) The maximum depth of detection is 2 cm from the surface of the skin. (2) ICG shows very high fluorescence intensity and just a small leak of ICG can contaminate the surgical field. (3) Quantitative evaluation of fluorescence is difficult at present. (4) A halogen

surgical lamp contains over 840 nm of wavelength light and must be turned off during fluorescence detection. Each of these problems can be solved if the RI method is used in combination with the ICG fluorescence method. However, we assumed an RI-free situation and suggest the following solutions: for issues (1) and (2), use of preoperative ultrasonography and marking of potential lymph nodes. This marking helps to estimate the possible SLN location when the lymphatic channel cannot be traced through the skin as far as the draining SLN. Thus, the incision line can be drawn at the estimated SLN position and the risk of damaging unnecessary lymph channels reduced. However, the location of SLNs in the neck region varies and fluorescence is difficult to detect through the skin when the SLN is located in a salivary gland or under the muscle. If we perform ICG method alone, we cannot find the precise position of the SLN through the skin and we have to make a longer incision line to trace the lymph flow. Therefore, SLN mapping in the neck region still requires the RI method.

The question of lighting has not been well discussed, but this is no small problem. The operation and fluorescence observation cannot be performed simultaneously under a conventional halogen lamp. Thus, repeated observation of fluorescence requires repeated lamp shutoff, which leads to extended operation time. On the other hand, our lighting system intercepts wavelength light of 810 nm or longer and provides sufficient

light intensity for operating without affecting the detection of ICG fluorescence (Fig-1C and D). To the best of our knowledge, there has been no report of use of this type of light source. We suggest that using this system can be introduced very easily and will provide shorter operating time for SLN biopsy

In conclusion, our ICG-FDS could be constructed at a reasonable cost and allowed us to visualize real-time lymph flow efficiently during surgery and to confirm the route of the lymph channels to the draining SLN SLN biopsy of the groin and axillae could be performed with this ICG-FDS combined with ultrasonography despite use of a radioactive agent Moreover, sufficient visible light intensity was obtained by using a light source with a short-wavelength pass filter concomitant with fluorescence detection. However, SLNs in the neck still require the RI method because lymph flow in this region varies and fluorescence is difficult to detect through the skin when drainage is into a node in a salivary gland or under the muscle. Despite such limitations, this system can be purchased at low cost and is easy to use. It may become a powerful method for detecting SLNs in the groin and axillary regions. However, owing to the limited number of patients enrolled in this study, further research is needed.

This work was partly supported by a Management Expense Grants from the

Government to the National Cancer Center (21S-7⑥).

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Table 1　Parts used in ICD-FDS

Parts name/parts no.	manufacturer	price(JP¥/US$)
LED/L760-04AU　(50 LEDs)	Ushio lighting, Tokyo, Japan	¥29,000/$320
CCD camera/ WAT-902H3 UL	Watec, Yamagata, Japan	¥37,000/$410
Lens/ YF4A-SA2B	Fujinon, Tokyo, Japan	¥8,000/$89
TV monitor/ MG-1503J	G-force, Tokyo, Japan	¥18,000/$200
Power supply/ PBA30F	Cosel, Tokyo, Japan	¥4,000/$45
Long-pass filter/ LX840	Asahi spectra, Tokyo, Japan	¥18,000/$200
Short-pass filter/ SIX810	Asahi spectra, Tokyo, Japan	¥18,000/$200
MPEG encoder/ VR100	BitBay, Tokyo, Japan	¥20,000/$222
Others (connectors, cables,PVC tubes)		¥15,000/$167
	Total	¥138,000/$1534

Table 2　Characteristics of the sixteen patients

Pt No.	Primary tumor / location	SLN location (*: meta(+))	SLN detection through skin	Identified SLN Blue dye	RI	ICG
1	AC/ lt lower abdomen	lt groin	×	2	N.D	2
2	SCC/ lt 5th toe	lt groin	○	0	3	3
3	AC/ rt mid abdomen	rt axilla	○	2	2	2
		rt groin	○	1	1	1
4	MM/ rt sole	rt groin	○	1	1	1
5	SCC/ vulva	**rt groin***	○	2	2	2
		lt groin	○	1	1	1
6	MM/rt thumb	rt axilla	○	1	1	1
7	EMPD/ vulva	rt groin	○	1	0	1
		lt groin	○	1	1	1
8	MM/ lt temporal	lt retroauricular	○	2	2	2
		lt parotid	×	1	1	1
		lt mid-jugular	×	1	1	1
9	EMPD/ vulva	**lt groin***	○	1	N.D	1
		rt groin*	○	1	N.D	1
10	EMPD/ scrotum	**rt groin***	○	2	2	2
		lt groin	○	1	1	1
11	MM/ rt buttock	**rt groin***	○	3	N.D	3
12	EMPD/ vulva	rt groin	○	2	2	2
		lt groin	○	1	1	1
13	MM/left hand	lt axilla	○	2	1	2
14	MM/ left nose	lt submandibular	×	1	1	1
		rt submandibular	×	0	1	1
15	EMPD/ vulva	lt groin	○	2	2	2
		rt groin	○	1	1	1
16	SCC/lt leg	lt groin	○	1	1	1

AC: adenocarcinoma, SCC: squamous cell carcinoma, MM: malignant melanoma, EMPD: extramammary Paget's disease, rt: right, lt: left, mid: middle, N.D: not done
○: Yes, ×: No

Fig. 1

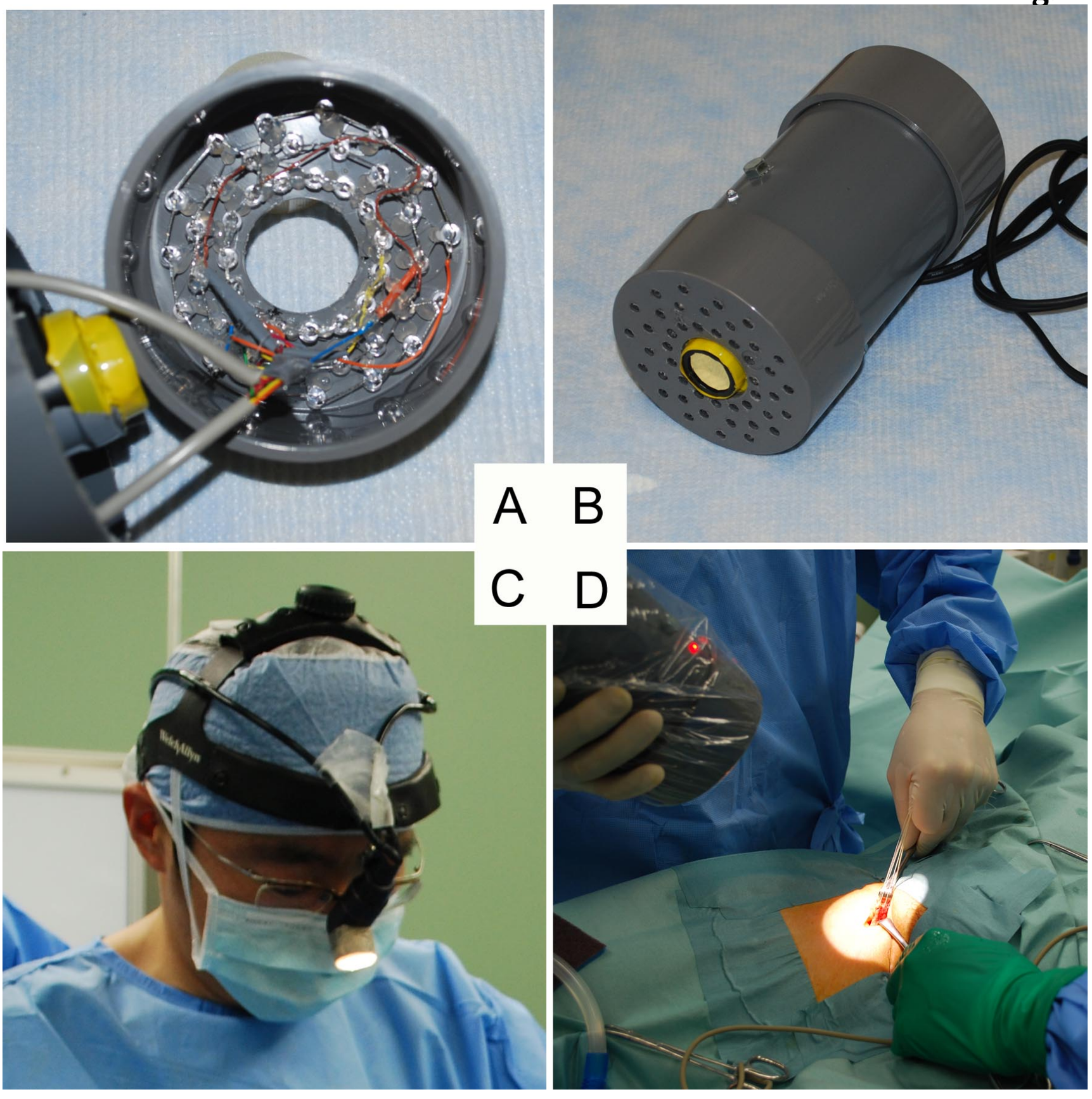

Fig. 2

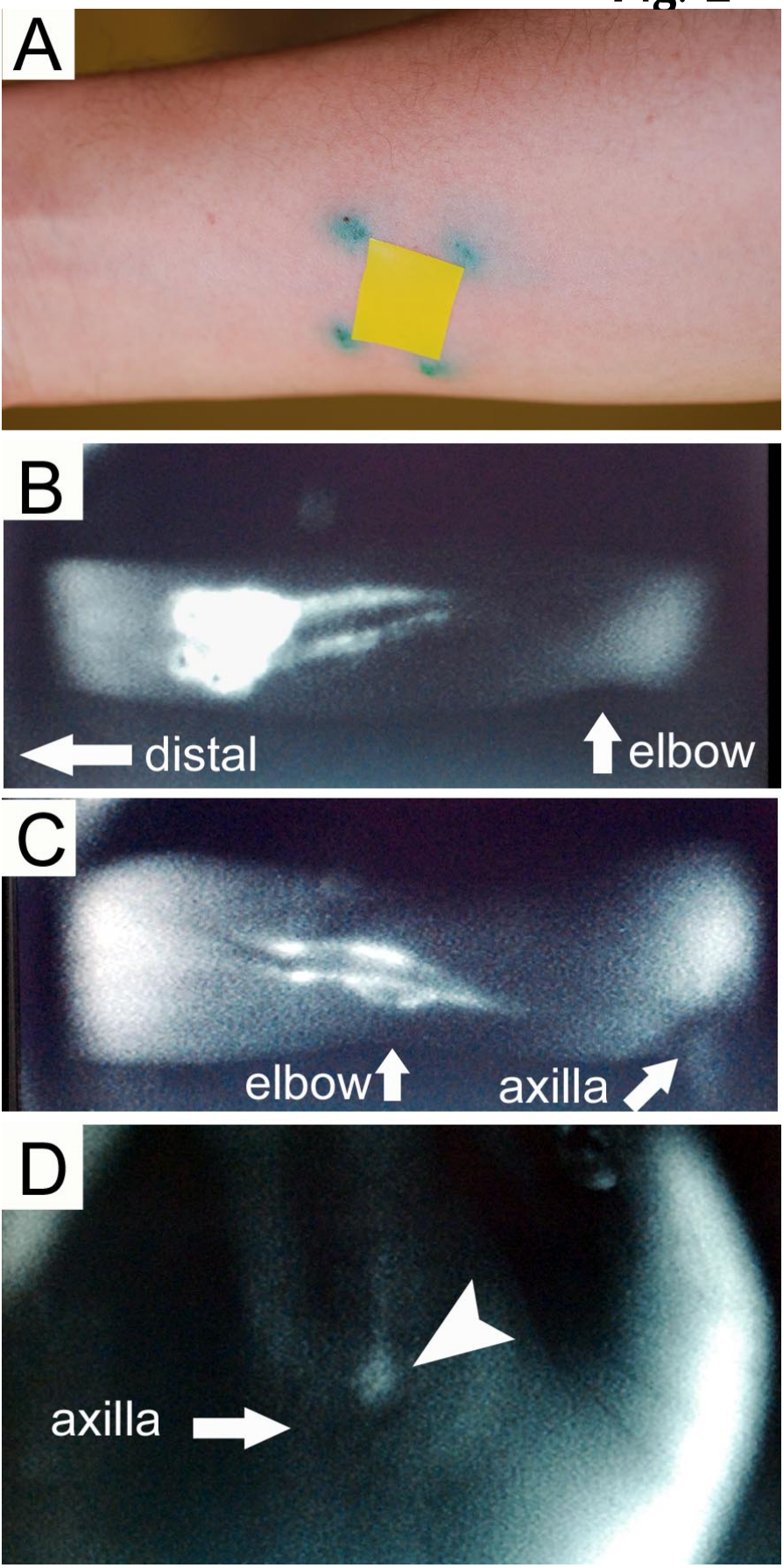

Fig. 3

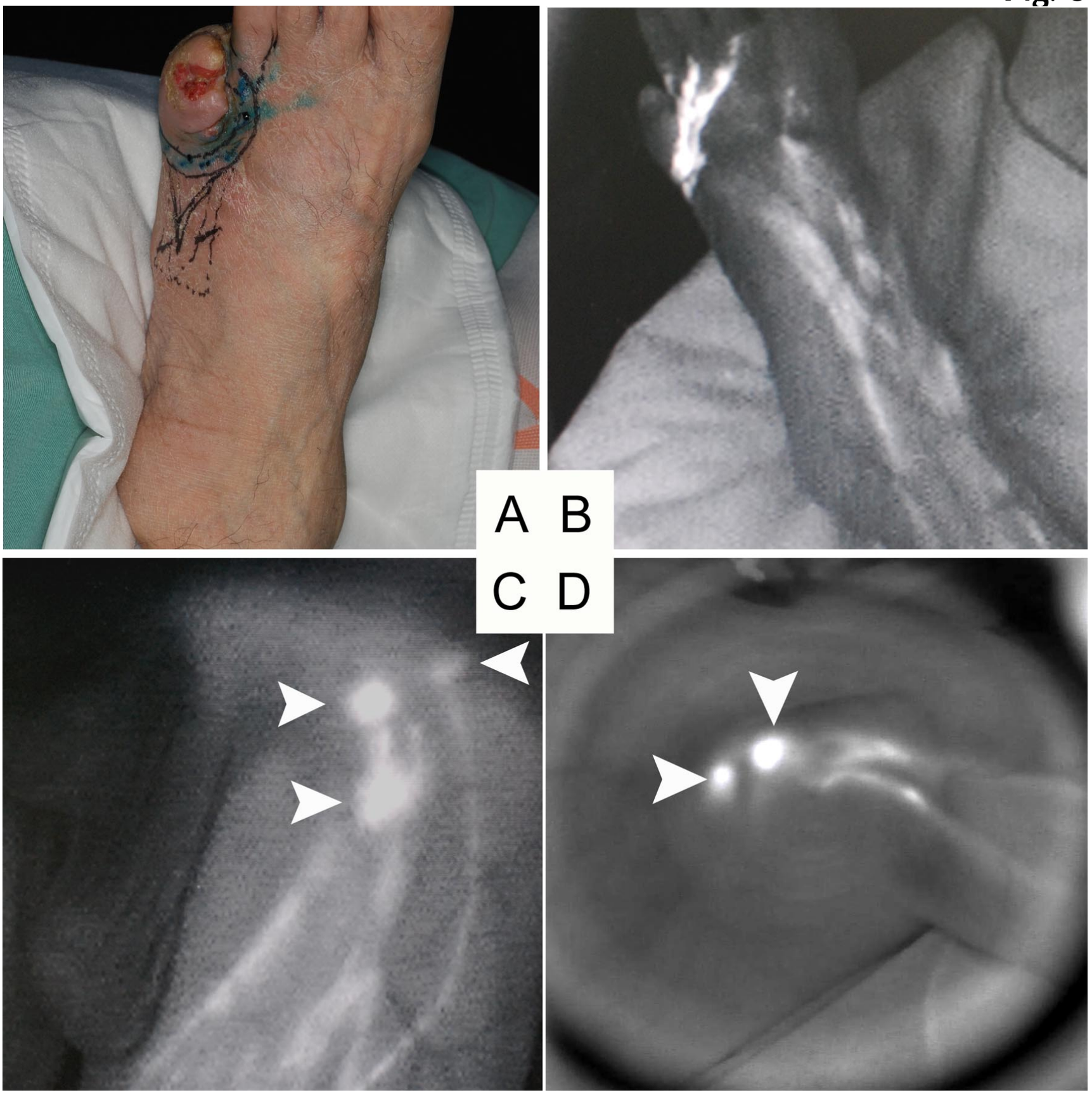

Fig. 4

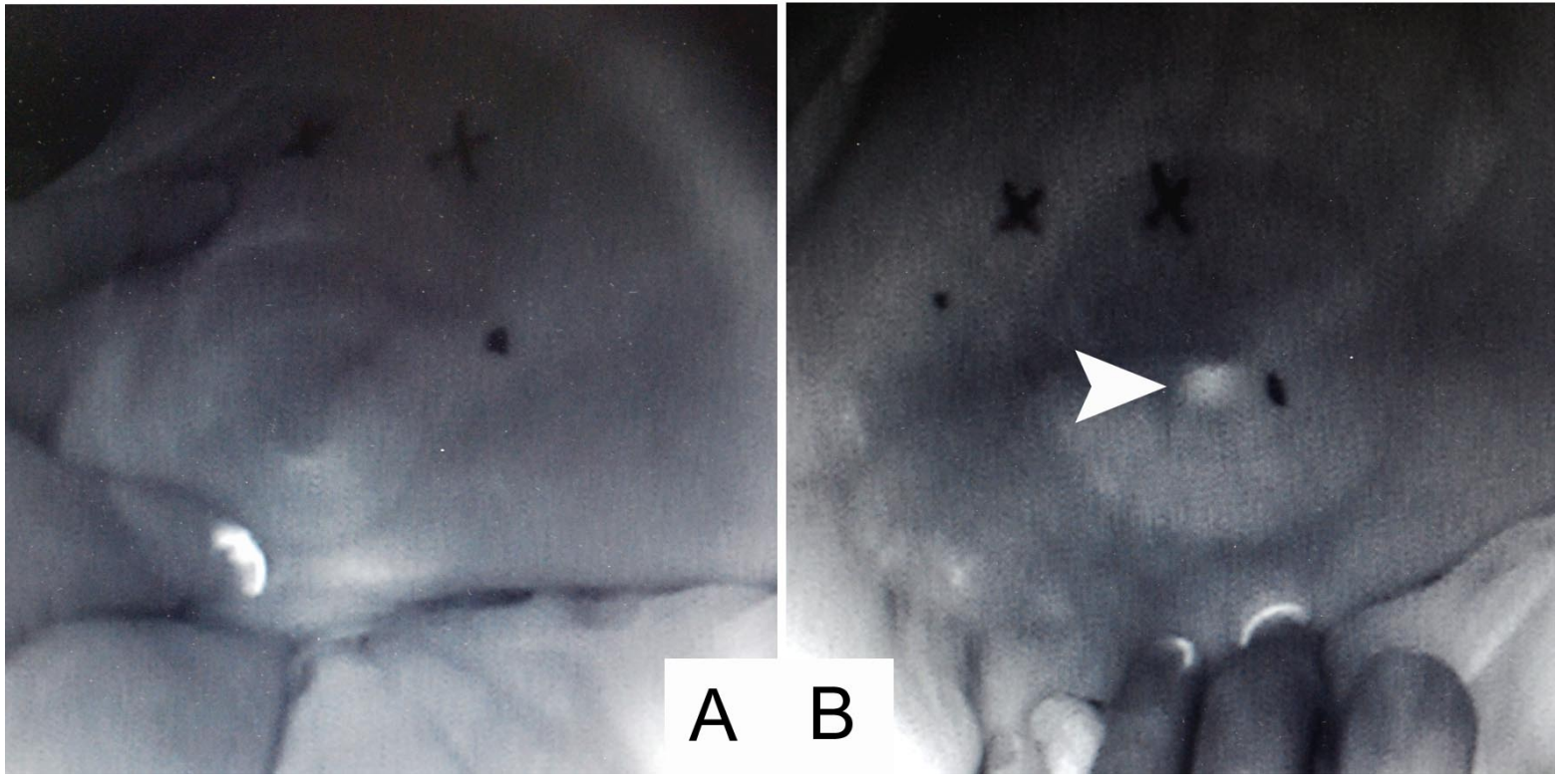

Fig. 5