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Development and validation of a custom made indocyanine green fluorescence lymphatic vessel imager

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Abstract. Lymphoedema is a chronic progressive condition often producing significant morbidity. An in-depth understanding of an individual's lymphatic architecture is valuable both in the understanding of underlying pathology and for targeting and tailoring treatment. Severe lower limb injuries resulting in extensive loss of soft tissue require transposition of a flap consisting of muscle and/or soft tissue to close the defect. These patients are at risk of lymphoedema and little is known about lymphatic regeneration within the flap. Indocyanine green (ICG), a water-soluble dye, has proven useful for the imaging of lymphatic vessels. When injected into superficial tissues it binds to plasma proteins in lymph. By exposing the dye to specific wavelengths of light, ICG fluoresces with near-infrared light. Skin is relatively transparent to ICG fluorescence, enabling the visualization and characterization of superficial lymphatic vessels. An ICG fluorescence lymphatic vessel imager was manufactured to excite ICG and visualize real-time fluorescence as it travels through the lymphatic vessels. Animal studies showed successful ICG excitation and detection using this imager. Clinically, the imager has assisted researchers to visualize otherwise hidden superficial lymphatic pathways in patients postflap surgery. Preliminary results suggest superficial lymphatic vessels do not redevelop in muscle flaps. [DOI: 10.1117/1.JBO.20.6.066003]

Keywords: excitation; fluorescence; wavelength; lasers; lymphoedema; lymphatics; indocyanine green.

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1 Introduction

The lymphatic system has important immune system functions ensuring a healthy body homeostasis. Stasis in lymph drainage can occur as a result of congenital abnormality of the lymphatic system or as a result of secondary effects of cancer treatment. When lymph stasis is long term it will result in visible swelling, often called "chronic edema," but more accurately called "lymphoedema" due to the failed lymphatic drainage of the affected area in the body. Lymphoedema is a chronic progressive condition where the lymphatic fluid load exceeds the lymphatic system's transport capacity.[1] If left untreated, the fluid is replaced by fatty tissues and eventually by fibrotic tissues, both of which further compromise lymphatic function.

Posttrauma lymphatic response to a high-energy impact injury is both highly variable between patients and poorly understood as a whole. While edema, as a response to lower limb trauma, is a recognized phenomenon, it is not well understood when there is significant soft tissue injury accompanied by underlying bone injury. Methods of soft-tissue reconstruction in this patient group involve the introduction of vascularized tissue to promote wound healing and redevelopment of the skin–environment interface. This tissue includes variable amounts of muscle, epifascial fat and skin with an existing, well-developed vasculature. While the revascularization of the reconstructed area has been researched extensively, few studies have been conducted that target the understanding of the repair of lymphatic function.[2]

Discovery of lymphatic vessels as "white veins" is dated back to the early 15th century, but the lymphatic system has remained a mystery for quite some time.[3,4] Due to their thin delicate walls, their tendency to contract when touched, and their translucent appearance it has been challenging to visualize the lymphatic system in humans. In the 1950s, direct injection of a radio-opaque contrast agent allowed lymph vessel imaging to occur clinically.[4] Imaging, however, has remained invasive, often with mild radioactive contrast agents. In addition, current methods display poor spatial resolution as well as being costly and time consuming for both the patient undergoing the imaging and the radiologist.[5]

Indocyanine green (ICG) is a water-soluble tricarbocyanine dye. In the mid-1950s, ICG was introduced into diagnostic medicine for cardiac output measures, liver functioning, and ophthalmic angiography.[6] More recently (2005), its use for detecting sentinel lymph nodes in breast cancer patients was successfully explored by Kitai et al.[7] in Japan. Since then ICG has rapidly developed into a lymphatic imaging technique.

Due to its rapid binding to protein, high sensitive fluorescence properties, and low toxicity, ICG provides a minimally invasive method of lymph imaging. For superficial lymphatic imaging, ICG is injected into the intradermal layer temporarily creating high pressure in the interstitial space. It binds to protein

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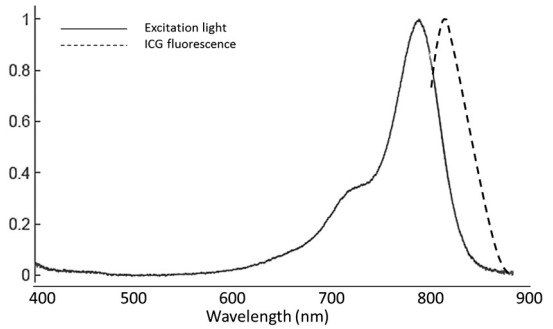

Fig. 1 Spectra of indocyanine green (ICG) excitation light and ICG fluorescence.[11]

(mostly albumin) present in the interstitial space, and in the normal process of draining the interstitial space the ICG is transported into the lymphatic vessels. The fluorescence intensity of ICG is dependent on the albumin concentration in the tissues and the presence of subcutaneous fat.[8,9]

On exposure of tissue to light, wavelength-dependent scattering and absorption occurs. In biomedical applications, wavelengths typically range from visible light to the near-infrared (IR) (700 to 1400 nm), where the tissue absorption is minimal. This allows a maximum amount of light to travel through the tissue.

The ICG fluorescence lymphatic vessel imager was designed to be used with PULSION Medical Systems® ICG, which has optimal ICG excitation between 700 and 830 nm and fluorescence between 760 and 870 nm[10] (Fig. 1).

Commercial ICG imaging systems did exist at the time of this study (Hamamatsu Photo Dynamic Eye®, Hamamatsu, Japan; SPY® Imaging System, Novadaq, USA; Fluobeam®, Fluoptics, France; and HyperEye Medical System®, Mizuho, Japan). These commercial systems were both cost-prohibitive and unavailable in Australia, which led to the development of the custom made imager reported here.

2 Materials and Methods

The ICG fluorescence lymphatic vessel imager was designed to be used in a clinical trial where ICG would be superficially injected into the subjects' skin near the toes of both feet to allow the uptake of the dye by the lymphatic system. The image system was designed to excite ICG with a fixed bandwidth light source (700 to 830 nm) and record real-time video of the resulting fluorescence (760 to 870 nm; near IR spectrum) as the dye traveled through the lymphatic system.

The custom made imaging system [Fig. 2(a)] consisted of an image head, positioning frame, trolley, and laptop computer with video capture software. The image head [Fig. 2(b)] comprised of a video camera, aspherical IR varifocal lens mounted on the camera, long-pass filter with custom mounting, eight laser diode flashlights, and a controlled white light source (itemized in Table 1).

Due to the small difference between the excitation light and emission peak wavelengths (Fig. 1), the system required a narrow bandwidth excitation source to prevent flooding of the detector. Laser diodes were selected with a center wavelength of 780 nm.

A video camera was used as the detector in this system. The camera contained a charge coupled device (CCD). The CCD contained an array of photosensitive capacitors and was sensitive from the visible to near-IR regions of the electromagnetic spectrum. An aspherical IR varifiocal lens was used to focus the incoming light onto the detector array and a long-pass filter was used to select the peak emission and avoid flooding of the

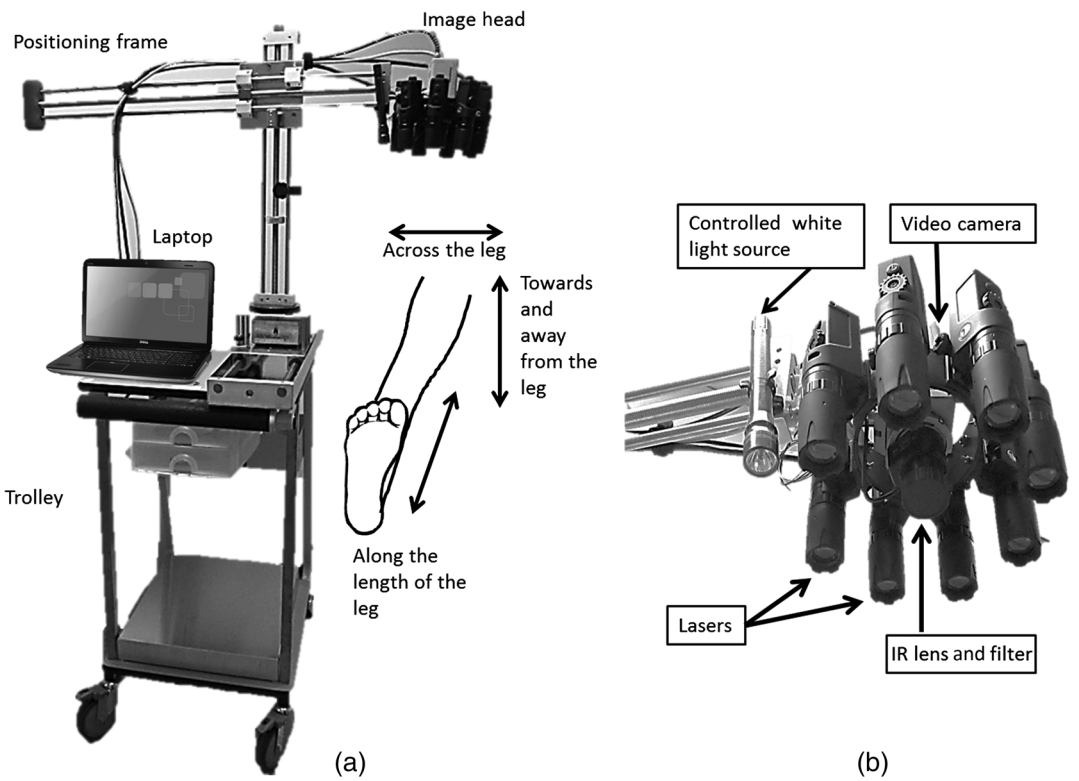

Fig. 2 (a) ICG fluorescence lymphatic vessel imager and (b) image head.

Table 1 Specification for the components used in the design of the indocyanine green (ICG) fluorescence lymphatic vessel imager.

Equipment	Specifications
Eight laser diode flashlights[12]	Pulsar, L-808S laser diode Wavelength: 780 nm Lens diameter: 22 mm Equivalent IR power: 250 mW Laser class: class 1 Range of power adjustment (min/max): 125 to 250 mW Range of beam divergence: 4.5 deg to 7 deg Custom supply made (6 V, 1.5 A nominal)
Video camera[13,14]	Panasonic, B/W WV-BP330 Series camera 1/3 in. CCD Scanning area: $4.9\,(H) \times 3.7\,(V)$ mm Scanning system: 2:1 interlace Video output 1.0 V [p-p] EIA composite 75W/BNC connector
Aspherical IR varifocal lens[15]	Daiwon Optical Co. Ltd., VIR3080AS Focal length: 3.0 to 8.0 mm Viewing angle: 44.1 deg to 118.7 deg diagonal Dimensions: 35 mm $\varnothing \times 48$ mm (L)
Long-pass filter[16]	Edmund Optics Pty Ltd., LP 850 nm Rejection wavelength: 200 to 835 nm Transmission wavelength: 865 to 1650 nm Diameter: 12.5 mm Transmission: $\geq$91% average Cut-on wavelength: 850 nm Cut-on tolerance: $\pm$1%
Video capture software[17]	VirtualDub Video capture/processing utility for 32- and 64-bit Windows platforms
White light source	Battery operated

detector. By utilizing a video camera, real-time fluorescence was captured. Software was used to convert the video into short segments and still images from which the lymphatic pathways were determined.

During operation of the ICG fluorescence lymphatic vessel imager, all external light sources were switched off to avoid flooding the detector and interference in the captured images. This, however, made it difficult for the operator to use the controls for focusing the camera and locating the point of dye injection on the foot. To assist, a controlled white light source [Fig. 2(b)] was used to provide gentle illumination in the area of interest prior to imaging without flooding the detector and corrupting the resultant image. Furthermore, the white light source was turned on intermittently during the imaging process to make the outline of the leg visible in the captured image (Fig. 3).

The specifications of the individual components of the system are outlined in Table 1.

The image head was mounted on an adjustable positioning frame (Fig. 2) allowing it to be moved in three planes during the procedure; distal to proximal (along the length of the leg), and laterally (to move across the leg and between left and right legs), as well as up and down (for focusing). This allowed the operator to position the image head at the start position and to smoothly move it when tracking the uptake and fluorescence of the dye traveling in the lymphatics of the lower leg. The complete system was mounted on a trolley to ease transportation to, and positioning within, examination rooms.

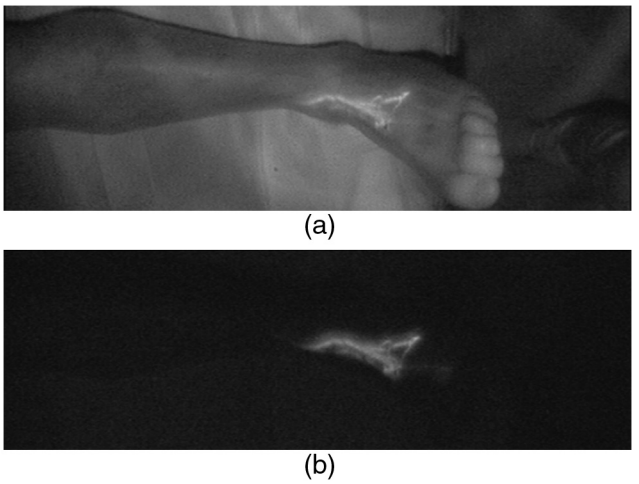

Fig. 3 (a) Captured image with the controlled white light source on. (b) Captured image with the controlled white light source off.

3 System Validation Trials

To test the functionality of the ICG fluorescence lymphatic vessel imager, trials were conducted on an animal model. Porcine hind legs were used due to their similarities with human skin. The hind legs from two culled 12-week old, 40 kg pigs were studied. The pigs were injected with 25,000 units of heparin and ketamine 4 min before culling.

For the animal trial, ICG freeze dried powder, obtained from PULSION Medical Systems, was diluted in water (5 mg/mL).[18] The solution was then injected between the hooves to mimic the intradermal dorsal side of the human foot. Once injected, the pig leg was massaged and the joints were mobilized to enhance the uptake of the dye into the lymph system. After massage, the image system was positioned 300 mm away from the site of interest, the imagers' excitation lasers were energized and images of fluorescing dye were recorded for 20 to 30 min. ICG fluorescence was detected and recorded immediately at the injection site and fluorescing superficial lymph vessels were detected and recorded 2 min post dye injection (Fig. 4).

4 Preliminary Clinical Trial Results

Following Royal Adelaide Human Research Ethics Committee approval (protocol 121123 approved on February 11, 2013), the ICG fluorescence vessel imager was used in a human clinical trial at the Royal Adelaide Hospital, Adelaide, Australia. Participants were recruited from a lower limb trauma database from the Department of Plastic and Reconstructive Surgery at the Royal Adelaide Hospital. All those who suffered severe lower limb trauma between 2009 and 2014 and who underwent reconstruction, with either free or locoregional flaps, were considered for recruitment. Clinical imaging of the lymphatic system was performed with the ICG fluorescence lymphatic vessel imager. Two intradermal injections of PULSION ICG dye (0.1 to 0.2 ml at 5 mg/mL concentration) on the dorsal side of each foot were sufficient for superficial lymphatic mapping. All measurements were repeated on each participant's nonaffected leg

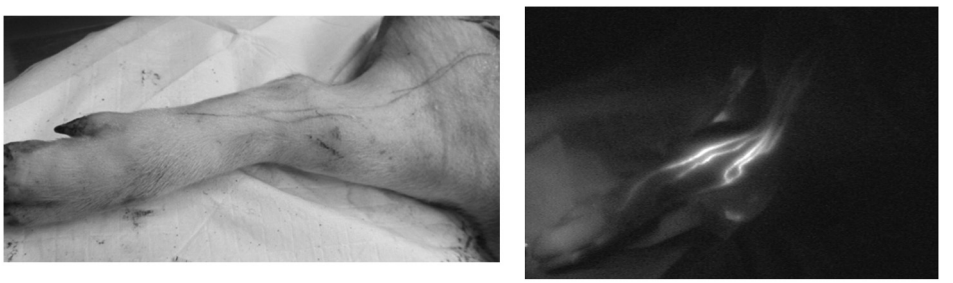

Fig. 4 (a) Hind leg of the pig with marked lymphatic pathway on the skin. (b) Fluorescence produced in the animal model highlighting the lymphatic vessels through the skin.

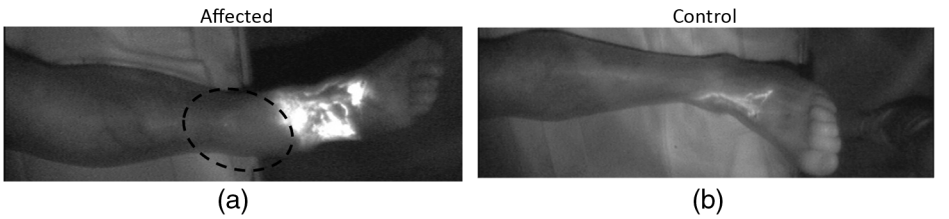

Fig. 5 (a) ICG fluorescence captured with the ICG fluorescence lymphatic vessel imager on the lower leg of a subject post muscle free flap reconstruction performed in 2013. The circle in the image highlights the region of reconstructive surgery. (b) Contralateral (control) leg for the same participant.

as a control. As of February 2015, 22 subjects had been recruited of which 18 were eligible for ICG imaging (2 females and 16 males). The mean time since reconstruction was 38 months (range: 2 to 62), the mean age at presentation was 47 (range: 25 to 72), and the mean BMI was 30.6 (range: 23.4 to 35.1).

Lymphatic pathways were imaged in all 18 subjects. Figure 5 shows typical images captured by the ICG fluorescence lymphatic vessel imager (the surgical site is denoted with a circle).

In most legs with reconstructed muscle flaps, fluorescence occurred in a large region distal to the flap [as shown in Fig. 5(a)], but fluorescence was not observed from within the flap.

5 Discussion

The ICG fluorescence vessel imager used laser diodes to excite ICG and induce fluorescence as they provide a narrowband light source. While a similar result could be achieved with a wideband light source and narrowband filter (general approach to fluorescence imaging before LEDs and laser diodes became commonplace), laser diodes represented a simpler and cheaper option with low energy and heat dissipation requirements. Due to the use of narrowband light sources in the design of the ICG imager, a need for background lighting to identify the outline of limbs and location of fluorescence was required. The controlled white light source was used for this purpose.

The detection of fluorescence from superficial lymph vessels within 2 min of dye injection into porcine legs (Fig. 4) indicated that laser diode flashlights can generate excitation light with sufficient intensity for fluorescence imaging applications, and a video camera can capture the fluorescence to produce suitable images to map lymphatic pathways.

It was evident from the preliminary clinical trials that the system was capable of allowing visualization of lymphatic pathways in both the controlled and affected limbs and by comparing these two, determinations of atypical lymphatic pathways were made. The clinical trial data concluded that in most cases no lymphatic flow from the distal extremity into the muscle flap was observed and that lymphatic fluid leaked out of the lymphatic vessels into surrounding tissues. This latter observation represented dermal backflow patterns around the flap area and these patterns are similar to those published by other groups utilizing commercially available imaging systems.[5,19–21]

ICG as a tool for lymphatic mapping is a novel technique and could prove to be useful in early diagnosis of lymphoedema and for the identification of the early stages of lymphatic dysfunction. The advantages of the ICG imaging technique are that it is not radioactive, it is minimally invasive, can be used for real-time imaging, and has better resolution compared to other lymphatic imaging techniques such as a lymphscintigram.[9]

While the ICG imager allowed visualization of lymphatic pathways, the quantification of images requires further investigation. Vessel depth and fatty tissues can result in scattering of the fluorescence which may lead to misinterpretation of the observed lymphatic flow and patterns.[22]

The development of this custom made system allowed ICG imaging to be accessible to a small research team; however, as costs drop, technology improves and ICG imaging is accepted as a diagnostic aid, commercial systems are anticipated to become more accessible.

6 Conclusion

The ICG fluorescence lymphatic vessel imager was manufactured as a custom made system which excited ICG and detected and recorded ICG fluorescence as it traveled within the lymphatic system of the lower leg. Preclinical and clinical trials demonstrated that the system was capable of imaging lymphatic vessels *in vivo*. The imager has enabled researchers to visualize the otherwise hidden superficial lymphatic system pathways in

flap reconstructed surgery patients. Improving the understanding of lymphatic regeneration after severe soft tissue trauma will increase awareness of the risk of lymphoedema in patients' postflap surgery and will help to improve treatment for this patient group.

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