

Review

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# The methodology of lymphatic anatomy studies in a cadaver model: an overview

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## Abstract

The lymphatic system is the area least investigated in the field of anatomical science. The major reason for this is the technical difficulty in identifying the lymphatics in the surrounding tissue in post-mortem specimens. As a result, the medical illustration masterpieces crafted by pioneer anatomists on the basis of cadaver dissections remain a vital component of current anatomical textbooks. Several innovative techniques were developed in the past to allow anatomists to distinguish the transparent lymphatic structures from their surroundings and enable thorough investigation of the lymphatic system in a cadaver model. This paper focuses on these techniques, including the current technique developed by the authors themselves.

**Keywords:** Anatomy, lymphatic system, cadaver, mercury, indocyanine green

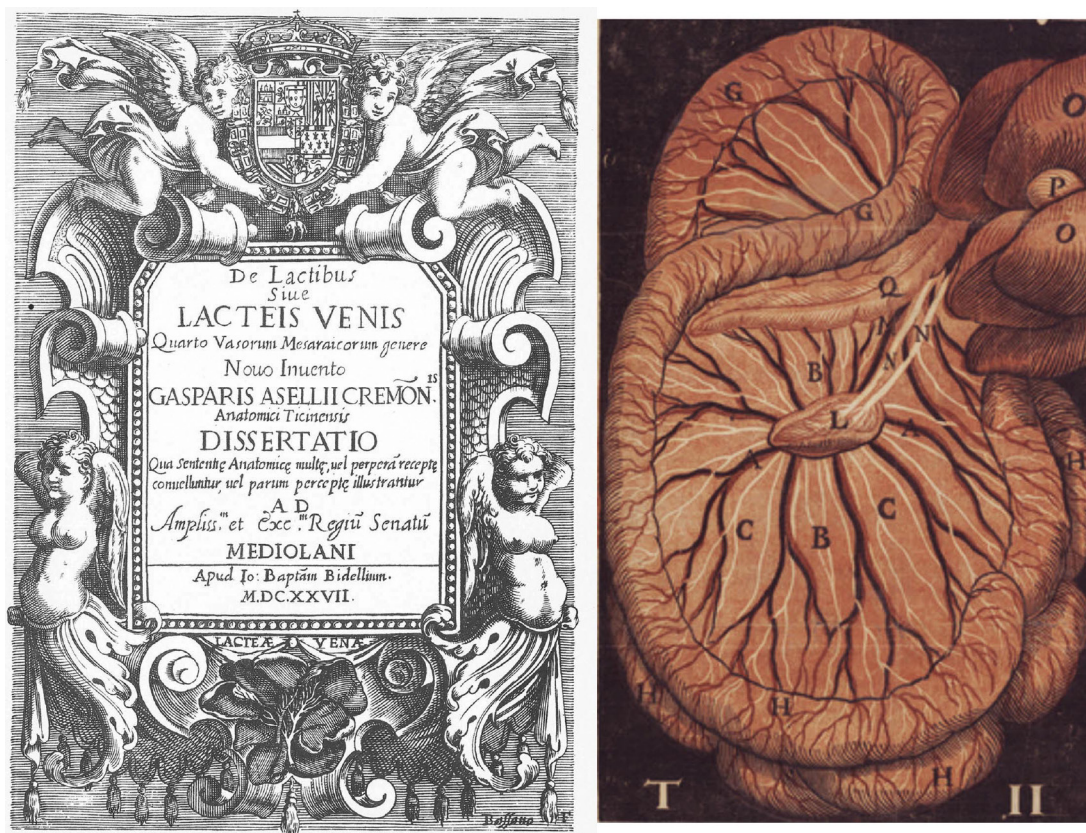
## INTRODUCTION

Since lymphangiography was introduced by Kinmonth<sup>[1]</sup> in the 1950s, several other imaging techniques have been developed to provide imaging of the lymphatic system in the clinical setting. Lymphoscintigraphy<sup>[2,3]</sup> is the current gold standard procedure, but indocyanine green (ICG) lymphography<sup>[4-6]</sup>, magnetic resonance lymphography<sup>[7,8]</sup>, and single-photon emission computed



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**Figure 1.** Aselli's<sup>[10]</sup> publication in 1627. This image shows white lacteal cords in the dog mesentery (right)

tomography (SPECT)/CT<sup>[9]</sup> are emerging imaging techniques to visualise the lymphatics. The tracers used for these examinations vary according to the procedure and are carried spontaneously via the lymphatic system after being injected into the skin or subcutaneous tissue. Understanding the precise anatomy of the lymphatic system is fundamental for providing a normal control view of the particular body region being examined that in turn enables doctors and researchers to identify changes in patients.

Despite the range of clinical imaging examination techniques, studies of lymphatic anatomy are very limited. The spontaneous transit of lymph fluid terminates in post-mortem specimens and valvular structures inside the lymphatic vessel are located at intervals of a few millimetres. These characteristics mean that retrograde injection from the proximal to the distal is not possible, so injections must be done from distal to proximal. In addition, the lymphatic vessels are transparent and lymph fluid is colourless as it contains no red blood cells, thus it is difficult for anatomists to distinguish lymphatic structure from the surrounding soft tissue. However, pioneer anatomists overcame these difficulties and created detailed medical drawings of the lymphatic system based on their dissections. These drawings continue to be a feature of current anatomical textbooks.

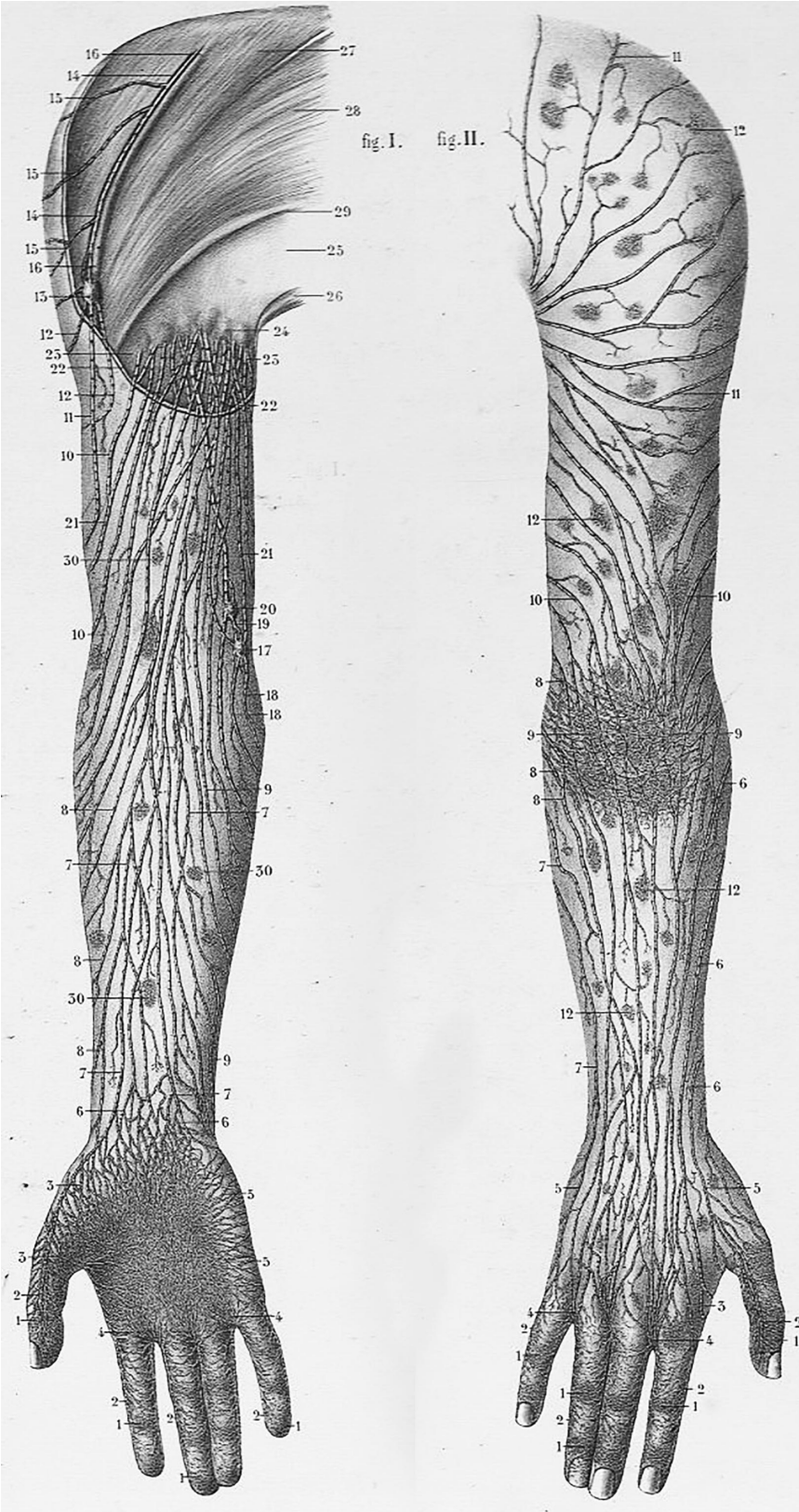
In this article, we provide an overview of the historical techniques used in the study of lymphatic anatomy and introduce our own contribution to this field.

## HISTORICAL REVIEWS

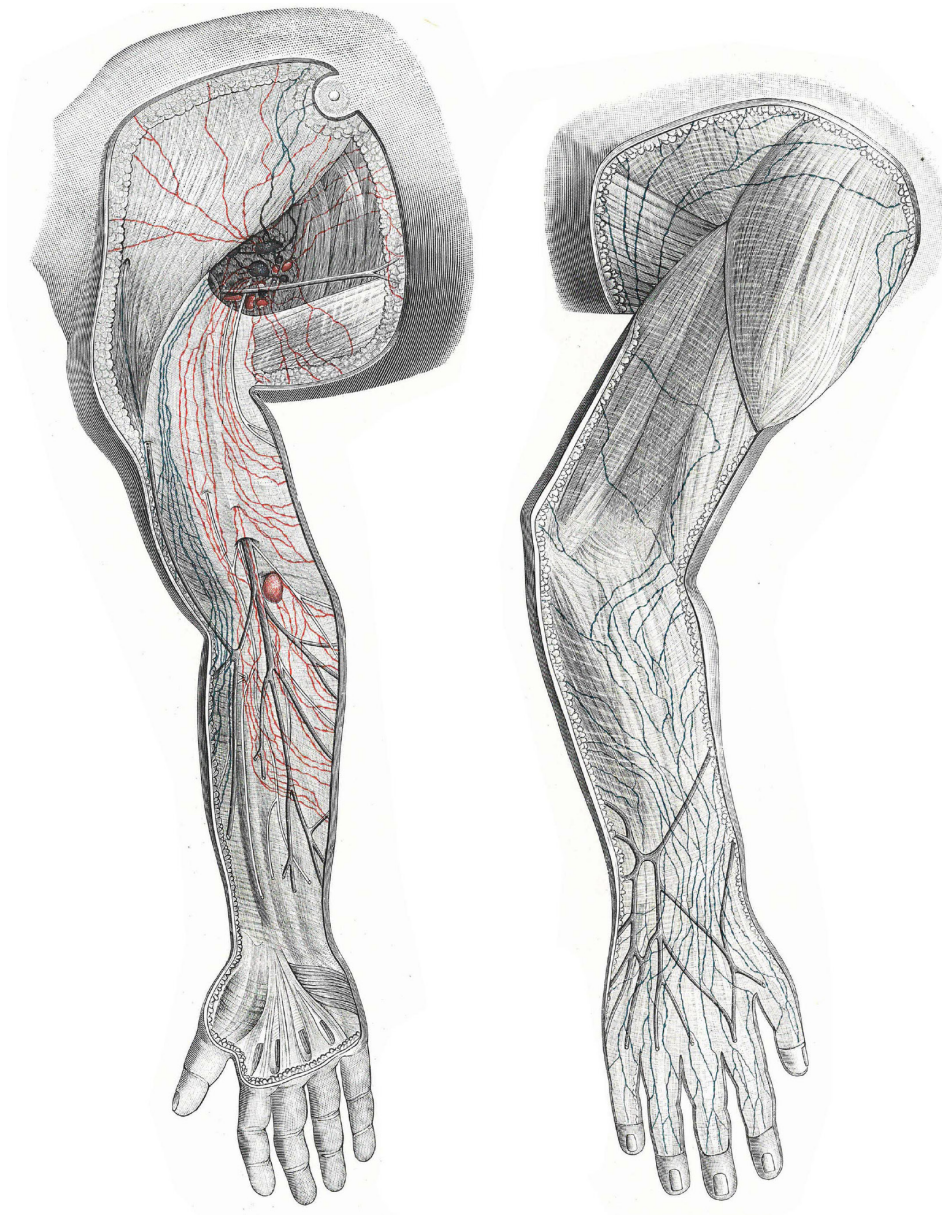
### Dissection of living animals - discovery of the lymphatic system

Discovery of the lymphatic system is credited to the Italian anatomist Aselli<sup>[10]</sup> [Figure 1]. When Aselli was asked by his colleague to demonstrate the recurrent nerve in a living dog, he noticed by chance that





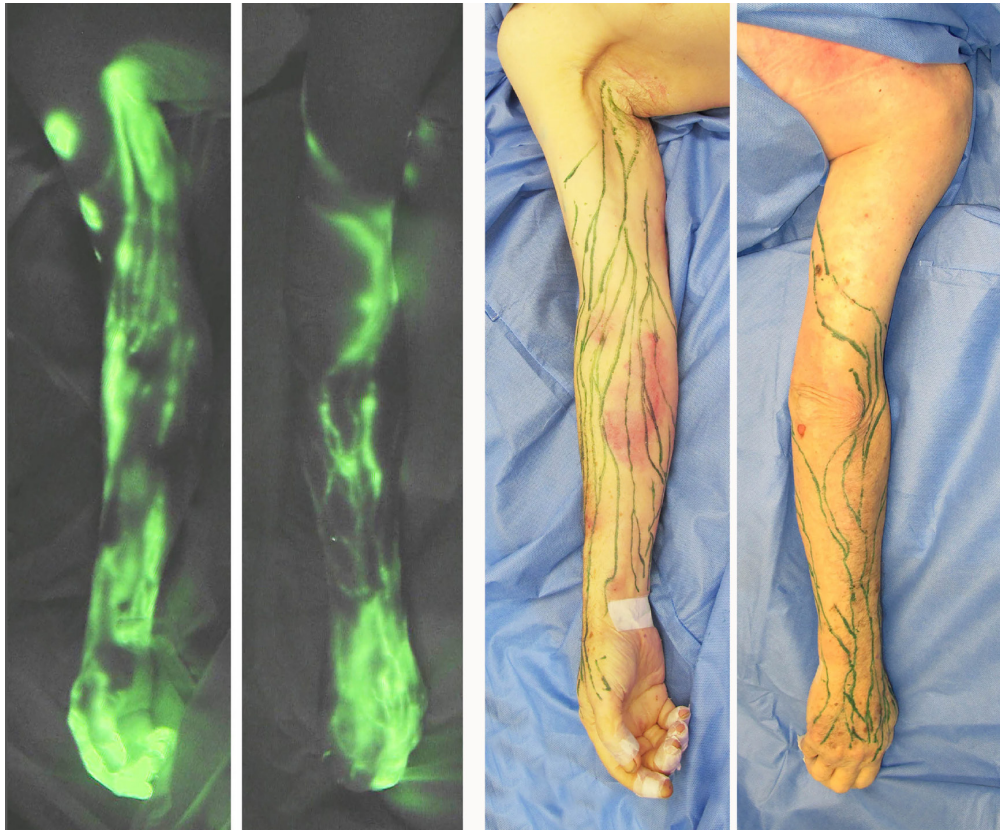
**Figure 3.** Illustration from Sappey's<sup>[19]</sup> book showing lymphatic vessels in the upper extremity identified using the mercury injection technique



**Figure 4.** Illustration from Bartels<sup>[24]</sup> book showing lymphatic vessels in the upper extremity identified using Gerota's method

Sappey<sup>[19]</sup> also used the mercury injection technique and published his findings in 1874 [Figure 3]. His book provided a comprehensive understanding of the human lymphatics, but his superb illustrations were more of artistic than scientific value. Sappey was probably the first anatomist to understand the idea of lymphatic territories defined by a watershed at the midline and a horizontal line crossing the umbilicus in the torso.

To date, anatomical findings with mercury injection were the mainstay of our understanding of the lymphatic system. Anatomists injected mercury directly into the lymphatic vessels or indirectly into the skin in cadaveric specimens using stretched glass tubes or fine needles. The excellent illustrations made by the early anatomists led to the belief that mercury was an ideal material for demonstrating the lymphatics, but it has several downsides. Firstly, mercury is a toxic substance and its use in anatomical investigation was discontinued in the early twentieth century due to concerns about health issues. Secondly, mercury



**Figure 5.** Indocyanine green lymphography image in the upper extremity in a fresh cadaver (left). Lymphatic mapping in the same specimen (right)

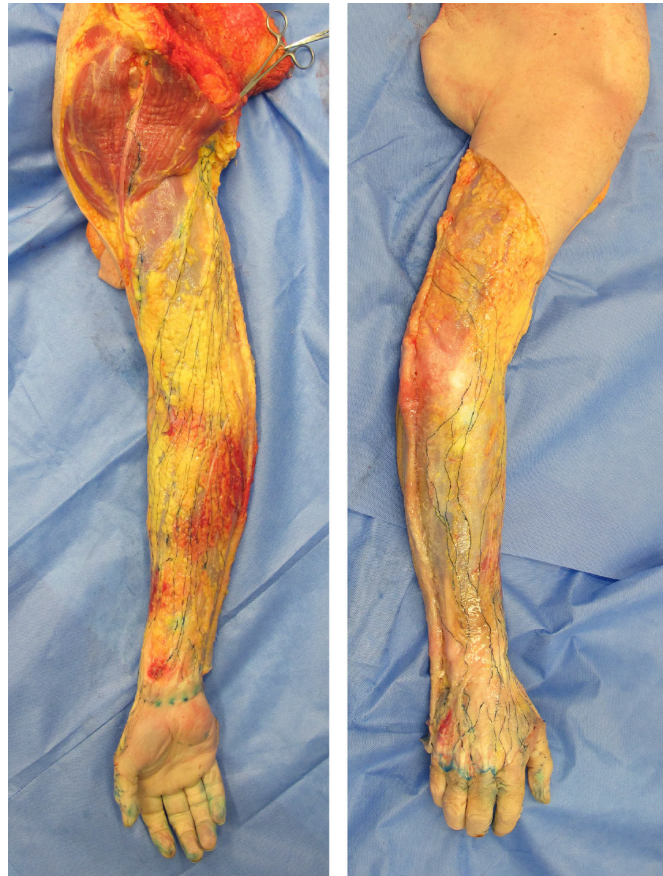
was an imperfect tool for identifying the lymphatics. A young male cadaver that had been studied using a mercury injection in the 18th century, possibly by Alexander Monro the Second (1733-1817), was stored at the University of Edinburgh<sup>[20]</sup>. When this cadaver was later submitted to radiographic investigation, the radiographs revealed that the mercury had entered not only the lymphatic vessels, but also the veins<sup>[21]</sup>. This finding suggests that the anatomists doing the illustrations must have spent a lot of time and effort in manually extracting the information about the lymphatics to create their drawings. Finally, the mercury injection technique was limited in the number of lymphatic vessels that could be identified in each specimen. Hence, Sappey needed to combine findings from multiple specimens to compose a single diagram.

Despite these several downsides of the technique, the painstaking efforts made by pioneer anatomists who used mercury injection succeeded in establishing the solid foundation of lymphatic anatomy for three centuries, and our current knowledge still relies heavily on their achievement.

### Dye injection

As a potential replacement for mercury injection, a dye injection method for demonstrating the lymphatics was developed by Gerota<sup>[22]</sup> in a technique known as Gerota's method. Gerota's original medium was composed of Prussian blue diluted with turpentine and ether and his method was used by Rouvière<sup>[23]</sup> and Bartels<sup>[24]</sup> and contributed to their publications [Figure 4].

While studies using mercury injection used mainly adult cadaver specimens, Gerota's method required the use of foetal and child cadavers. The reason for this is that the slippery nature of mercury allowed



**Figure 6.** Lymphatic vessels in the specimen in [Figure 5](#) shown using the microinjection technique

it to travel long distances inside the lymphatic vessels, but the dye used in Gerota's method could only travel much shorter distances. To overcome this limitation, anatomists began using smaller bodies, but the difficulty of acquiring a steady supply of foetal specimens became a barrier to anatomical study and was the major factor in the decline of lymphatic anatomy research in the twentieth century. Foetal studies using Gerota's method still continue at the Department of Anatomy at the University of Buenos Aires, where the method is combined with the Spalteholz technique to render tissues translucent<sup>[25]</sup>.

#### **Microinjection technique with hydrogen peroxide**

A new technique to investigate the lymphatic system in adult cadaver specimens without using mercury was developed by Suami *et al.*<sup>[26,27]</sup>. In contrast to arteries and veins that contain red blood cells, transparent lymphatic vessels cannot be identified with the naked eye post-mortem because they collapse. Initially, patent blue violet was injected into the cadaver to identify the lymphatic vessels. The dye succeeded in demonstrating some lymphatic vessels, but it also stained the surrounding tissue and prevented any further investigation. After this, hydrogen peroxide was used and found to be an ideal substance to distinguish the lymphatic vessels from the surrounding soft tissue without any contamination. When hydrogen peroxide is injected into the skin and subcutaneous tissue around the area of investigation, its reaction with tissue enzymes produces fine bubbles of oxygen. These bubbles inflate the lymphatic vessels so they can be identified under a surgical microscope. When the location of the vessels has been determined, a fine stretched glass tube or needle is directly cannulated into them and a contrast agent (a radiocontrast medium or dye) is injected to stain them with colour. Once coloured, the vessels can easily be dissected out from the soft tissue. The whole procedure is performed under a surgical microscope and therefore has become known as the microinjection technique. Compared to both the mercury injection technique and

Gerota's method, the microinjection technique requires more dextrous skills in manipulating lymphatic vessels under the microscope.

When a radiocontrast medium is selected as the contrast agent, the prospective radiographic images are similar to those produced by lymphoangiography in live subjects. Lymphangiography is an invasive procedure requiring a small surgery consisting of cutting the skin and cannulating a fine needle into a lymphatic vessel under local anaesthesia<sup>[1]</sup>. Usually, only one lymphatic vessel is cannulated. In contrast to lymphoangiography, the microinjection technique in cadaver specimens has no limitation on the number of lymphatic vessels that can be identified, so has the advantage of allowing the researcher to obtain a comprehensive picture of the lymphatics in each specimen. The disadvantage of the microinjection technique is that the contrast medium stops at the sentinel node, so further cannulation in the efferent vessel of the node is essential if the proximal areas are to be investigated. However, the invention of the microinjection technique enabled anatomists to undertake the study of lymphatic anatomy in adult cadavers and contributed to better lymphatic mapping of the body regions<sup>[28-31]</sup>.

### **Indocyanine green lymphography**

ICG lymphography was initially developed to map the sentinel lymph nodes for breast cancer treatment<sup>[32]</sup>. Its application was then expanded to provide imaging for the diagnosis of lymphoedema<sup>[4-6]</sup>. ICG is a water-soluble agent that emits near infrared-rays when it combines with protein in the human body. When ICG is injected into the skin, it automatically enters the lymphatic vessels. The infrared camera then scans the limb and captures the emitted signals, causing the vessels to glow green so that they can be identified in real time to a depth of 2 cm from the surface of the skin.

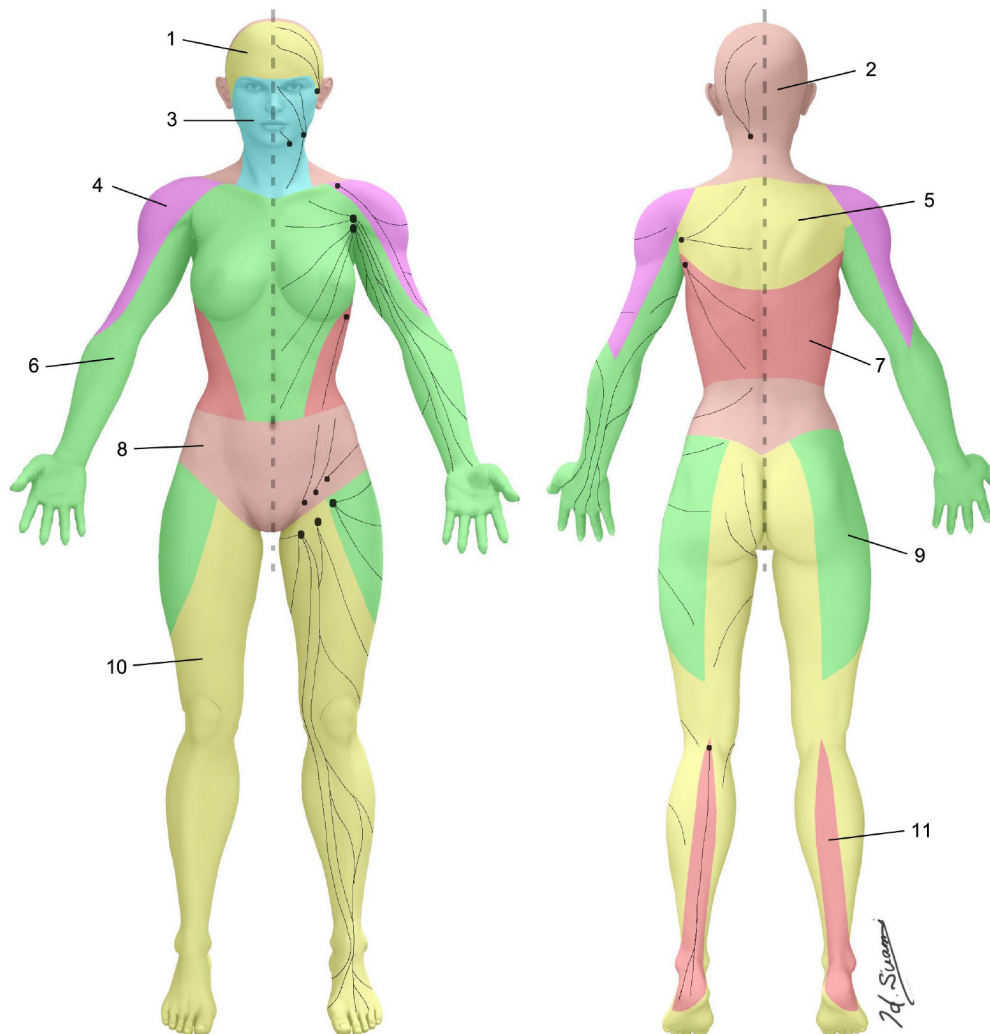
The authors injected ICG into fresh cadavers and confirmed that it was able to identify the lymphatic vessels in post-mortem specimens<sup>[33,34]</sup>. ICG lymphography was found to be consistent in identifying the lymphatics and the dye was able to travel long distances from the injection site if fresh, non-frozen cadavers were used. The technique has demonstrated several advantages in anatomical study in a cadaver model. Firstly, lymphatic vessels can be identified without a skin incision, so ICG injections do not disrupt the embalming process to follow and the bodies can be reutilised for anatomical dissection after study of the lymphatics. Secondly, ICG mapping has helped make the microinjection technique more effective, as it provides a more efficient way of locating the lymphatic vessels than injecting hydrogen peroxide, the most time-consuming part of the process. Finally, it has enabled fast mapping of the course of lymphatic vessels and allowed the capture of imaging data from multiple cadaver specimens<sup>[35]</sup>.

### **AUTHOR'S CURRENT METHOD**

Finally, this paper briefly introduces the specific method we currently use for anatomical investigation of the lymphatic system in a cadaver model. Cadaver specimens are obtained from the institutional willed body program with appropriate approval for their use in scientific investigation. Fresh, non-embalmed and non-frozen specimens are ideal, but previously frozen and thawed specimens can also be used. ICG (Verdye 25 mg, Diagnostic Green GmbH, Germany) is mixed with 20 mL of saline. Taking the investigation in an upper extremity as an example, 0.1 mL doses of ICG solution are injected intradermally into the sides of fingers and several spots in the anterior wrist. After a few minutes of massage at the injection sites, the ICG dye starts entering the lymphatic vessels. Inside the lymphatic vessel, the dye is moved from the distal to proximal by massaging the skin in an axial direction. If the specimen is within a few days post-mortem, the ICG moves all way to the axillary nodes and the course of the vessels is demonstrated with the infrared camera (Photodynamic Eye Neo II, Hamamatsu K.K., Japan). A marker pen is used to mark the lymphatic vessels on the surface of the skin [Figure 5].

If the specimens are needed for further dissection and for use in an anatomical teaching workshop, the microinjection technique is then applied<sup>[33]</sup>. Five per cent hydrogen peroxide with/without dye is injected





**Figure 7.** Lymphosomes of the body. The lymphatic territories are demarcated according to their corresponding lymphatic basins: (1) temporal; (2) occipital; (3) submental; (4) subclavicular; (5) subscapular; (6) lateral axillary; (7) pectoral; (8) superior inguinal; (9) lateral inguinal; (10) inferior inguinal; and (11) popliteal. (Reproduced with permission of Suami<sup>[37]</sup>, 2018)

around the lymphatic vessels identified by the ICG mapping to inflate them for cannulation. The inflated vessels are easily identified in the subcutaneous tissue below the area that has been marked. A 30 G needle connected to an extension tube and 1 mL syringe is set with a micromanipulator (UMM-3FC and UM-1PFC, Narishige Group Co., Japan) and cannulated into the lymphatic vessel. A coloured substance with/without a radiocontrast medium is injected into the lymphatic vessels by manually pumping the 1 mL syringe. After removing the skin just above the stained lymphatic vessels, the lymphatic course is traced until the vessels reach their corresponding lymph nodes [Figure 6].

The combination of ICG lymphography and the microinjection technique works effectively in fresh adult cadavers. We applied this technique to map the lymphatics and found that the skin can be demarcated into groups of lymphatic vessels that connect to the same regional nodes. The author coined the word “lymphosome” to describe these separate lymphatic territories [Figure 7]<sup>[36,37]</sup>.

Significant anatomical changes occur in lymphoedema<sup>[38-40]</sup>. Understanding the normal anatomy of the lymphatics is essential to allow doctors and researchers to distinguish how lymphatic structures altered by lymphoedema differ from the original condition. New imaging techniques continue to be developed and

provide new types of lymphatic images. Physicians need to be able to interpret these images accurately to specify the pathology of lymphatic dysfunction, and anatomical study of the lymphatics is essential to provide the required baseline information.

## CONCLUSION

This paper focuses on the techniques that have enabled anatomists to investigate the lymphatic system in cadavers over the past several hundred years. Mercury injection was the mainstay of lymphatic study for many years and our current knowledge still largely depends on findings from more than 100 years ago. New imaging techniques are being developed in the clinical setting, and anatomical research needs to be updated to incorporate these new techniques to provide further information about the lymphatic system.

## DECLARATIONS

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### Authors' contributions

Study concept and data acquisition: Suami H

Data interpretation and manuscript drafting: Suami H, Shinaoka A

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

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