Technological Advances in Lymphatic Surgery: Bringing to Light the Invisible

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Summary: Lymphatic surgery has become an integral and flourishing component of the field of plastic surgery. The diversity of ongoing technological innovations in perioperative imaging, including intraoperative dyes and cameras, allows plastic surgeons to work at the supermicrosurgical level. This study aims to highlight innovations that have shaped and will continue to revolutionize the perioperative management of the lymphatic surgery patient in the future. As additional advances emerge, we need a systematic and objective way to evaluate the efficacy and clinical integration readiness of such technologies. Undoubtedly, these technologies will help lymphatic surgery trend toward increasing objectivity, which will be critical for continued evolution and advancement. (*Plast. Reconstr. Surg.* 143: 283, 2019.)

With the advent of recent technologies, plastic surgeons are more adept at supermicrosurgery, as is evidenced in functional lymphatic surgery. The history of microsurgery dates back to the turn of the twentieth century, when Carrel and Guthrie performed the first replantation of visceral organs.[1,2] Buncke and Schulz pioneered the first microsurgical procedure in plastic surgery with the anastomosis of a 1-mm vessel in a rabbit ear reimplantation in 1966.[3] The first paraumbilical perforator flap was described by Koshima and Soeda in 1989, introducing a new era of flaps based on cutaneous perforators.[4–6]

These advances serve as the stepping-stone to the emergence of supermicrosurgery, which has been previously defined as a perforator-to-perforator anastomosis of less than 0.8 mm.[7] This technique, although reported frequently with regard to perforator flaps[6] and fingertip replantation,[8] is most commonly referenced when discussing functional surgical procedures for lymphedema.[9] The two most common techniques in the surgical treatment of lymphedema are vascularized lymph node transfer and lymphovenous anastomosis.[10] In the late 1960s, lymphaticovenous anastomosis emerged as a potential treatment for chronic lymphedema.[11–13] Similarly, almost a decade later, in 1982, the first clinical description of lymph node transfer for the treatment of lower extremity lymphedema was reported.[12]

In 2009, the field of lymphedema prevention was born when Campisi and Boccardo introduced the lymphedema microsurgical preventative healing approach. In this approach, the risk of lymphedema development is reduced by performing lymphaticovenous anastomoses (lymphovenous anastomosis) between arm lymphatics and collateral branches of the axillary vein at the time of lymphadenectomy.[14] This technique has significantly reduced the incidence of lymphedema to 4 percent at 4-year follow-up.[15] This review aims to highlight innovations that have shaped and will continue to revolutionize the perioperative management of the lymphatic surgery patient in the future.

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Table 1. Perioperative Lymphatic Imaging Technologies

Technology	Contrast	Penetration Depth	Radiation	Depth Resolved	Acquisition Time	Notes
Magnetic resonance	T2 or gadolinium	Whole body	None	Yes	30–100 min	Great three-dimensional resolution; FDA safety concerns over gadolinium toxicity buildup; slow; best for preoperative investigation
Computed tomography	Iodinated tracer	Whole body	Yes	Yes	20–60 sec	Great three-dimensional resolution; high radiation exposure; fast; best for preoperative investigation
Near-infrared fluorescence	Fluorophore	2 or 4 cm*	None	No	10–1000 msec	Low-cost; nonionizing; fast; modest depth penetration; best for intraoperative guidance or superficial investigation

FDA, U.S. Food and Drug Administration.
*4 cm depth achievable with intensified detector.

PERIOPERATIVE TECHNOLOGY

Imaging of the lymphatic system is critical to the lymphatic surgeon to obtain anatomical targets for intervention. The challenge of this endeavor rests on the size of the lymphatics, which ranges from 90 μm peripherally to 2 to 4 mm centrally at the thoracic duct. As a means of comparison, although the arterioles are 5 μm in diameter, centrally, the aorta measures 2 to 3 cm, thereby making the arterial system up to 10 times larger than the lymphatic tree depending on the anatomical level of comparison. Current available imaging techniques used to diagnose and stage lymphedema include nuclear lymphoscintigraphy, indocyanine green lymphography, magnetic resonance lymphangiography, and computed tomography (Table 1).

INDOCYANINE GREEN LYMPHANGIOGRAPHY

Indocyanine green lymphangiography was first used for lymphedema evaluation in 2007 by Ogata et al.[16,17] This technique uses a near-infrared fluorescent tracer, mainly indocyanine green, injected intradermally to identify lymphatic channels and patterns of lymph flow within minutes of injection. In addition to reliably detecting lymph flow abnormalities as in lymphoscintigraphy, indocyanine green lymphangiography offers the advantages of real-time, continuous, high-resolution, and high-sensitivity visualization of the lymphatic system without the need for ionizing radiation.[16,18,19] As near-infrared light is invisible, there is no staining of the surgical field as with blue dyes, and the class of chemicals to which indocyanine green belongs has an excellent safety profile.[18–25] Premixing indocyanine green with human serum albumin increases hydrodynamic diameter and preference for the lymphatic system, and thus improves fluorescence kinetics and quantum yield (brightness).[25,26]

Indocyanine green lymphangiography is particularly important in the early diagnosis and staging of lymphedema. Yamamoto et al. described two main patterns of indocyanine green findings: linear, corresponding to normal; and dermal backflow, representing pathologic state.[24] The dermal backflow pattern can be subdivided into splash, stardust, and diffuse patterns and correlates with progression of lymphedema severity (Fig. 1).[22,24] (**See Video, Supplemental Digital Content 1**, which shows preoperative indocyanine green lymphangiography for operative planning demonstrating both stardust and linear patterns in the affected extremity, ***http://links.lww.com/PRS/D204***.) A splash pattern is the earliest finding of asymptomatic lymphedema, which is critical for early detection and intervention.[21] In addition, dynamic indocyanine green lymphography details lymph pump function and circulation by means of indocyanine green velocity and transit time, offering a comprehensive assessment and staging of lymphedema.[23]

The all-optical instrumentation that indocyanine green angiography requires results in a relatively inexpensive, noninvasive, safe, and real-time imaging modality. The system requires a light source such as a narrowband laser, a light-emitting diode or broadband source with excitation filter to excite the fluorophore of interest (indocyanine green, approximately 750 to 780 nm), an emission filter to block the excitation light and to pass fluorescence (indocyanine green, approximately 800 to 860 nm) through the collection optics, and a detector such as a charged-coupled device or a complementary metal oxide semiconductor. These techniques use widefield illumination and do not require scanning of the illumination. This allows for penetration depths of 2 cm[27,28] in

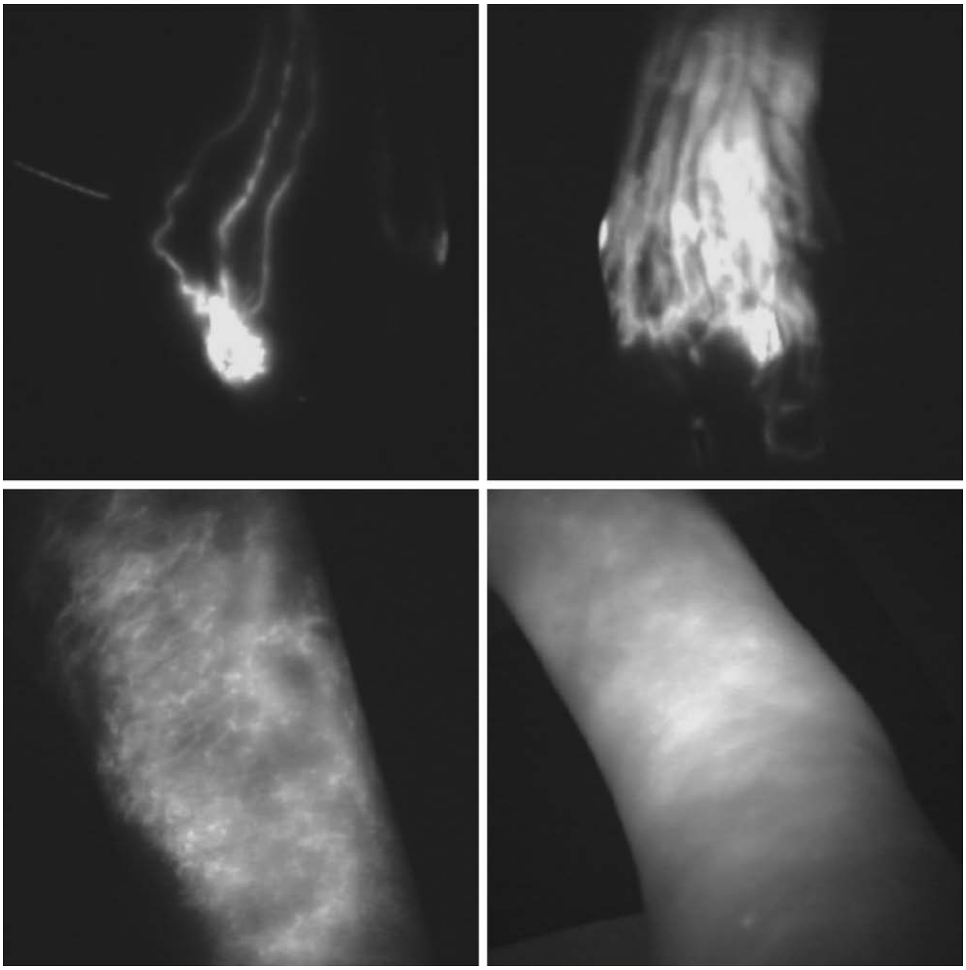

Fig. 1. Indocyanine green fluorescence patterns in patients with lymphedema. Linear patterns are typically associated with preserved lymphatic function (*above*, *left*). Dermal backflow patterns consist of splash (*above*, *right*), stardust (*below*, *left*), and diffuse patterns (*below*, *right*), corresponding to progression of disease. Reprinted with permission from Bao Tran B, Singhal D. Clinical diagnosis of lymphedema. In: Chen H-C, Ciudad P, Chen S-H, Tang Y-B. eds. *Lymphedema: Surgical Approach and Specific Topics*. Oxford, UK: Elsevier. 2018:22.

Video 1. Supplemental Digital Content 1 shows preoperative indocyanine green lymphangiography for operative planning demonstrating both stardust and linear patterns in the affected extremity, ***http://links.lww.com/PRS/D204***.

standard detection and up to 4 cm for intensified detection.[29]

Several systems have been developed for lymphangiography. Devices such as the Photodynamic Eye (Hamamatsu Photonics K.K., Hamamatsu, Japan) fluorescence imaging system,[30–33] HyperEye Medical System (Mizuho Medical, Tokyo, Japan),[34] FLUOBEAM (Fluoptics, Grenoble, France),[35] and Lab-FLARE Model R1 Open Space Imaging System (Curadel, Marlborough, Mass.) are commercially available and have been widely used for various lymphatic mapping techniques. Readers are directed to recent reviews of molecular imaging systems for more details.[36,37]

Currently, the only U.S. Food and Drug Administration–approved fluorescent tracer is indocyanine green, which tends to wash out rather quickly because of its particle size. Next-generation tracers have been developed to yield higher optical intensity and longer washout time.[38] IRDye 800CW is an indocyanine-type near-infrared fluorophore that is four-fold brighter than indocyanine green. Once conjugated to cyclic albumin-binding domain, IRDye800-cABD not only is optically enhanced but also has preferential affinity to the lymphatic system.[39] IRDye 800CW is also coupled with colloidal albumin to achieve prolonged lymphatic retention time.[40] Similarly, mannosylated liposome-encapsulated indocyanine green has a higher ultraviolet absorbance spectrum and fluorescence intensity and increased specificity of uptake and retention in macrophages, which are found in high concentration in lymphatic tissue, making it a good candidate contrast agent for the lymphatic system.[38]

NUCLEAR LYMPHOSCINTIGRAPHY

Lymphoscintigraphy, which can be performed with radiotracers, blue dye, or both, is considered the gold standard for functional evaluation of the lymphatic system.[41] The exciting development of new radiotracers has the potential to further the field of lymphatic surgery. Specifically, in the United States currently, filtered technetium 99–sulfur colloid is the most commonly used tracer.[41] A relatively new U.S. Food and Drug Administration–approved receptor-targeted radiotracer, technetium 99–tilmanocept (Lymphoseek; Navidea, Dublin, Ohio), has been used for mapping and localization of lymph nodes draining breast cancer, melanoma, and solid tumors.[42] The novelty of this tracer is that it preferentially accumulates in lymphatic tissue by selectively targeting and binding to CD206 receptors on the surface of macrophages and dendritic cells, which are found in high concentration in lymphatic tissue.[42] Technetium 99–tilmanocept exhibits other distinct advantages over radiocolloids, including rapid clearance at the injection site[43] and less postinjection pain at the injection site.[44] Given the affinity of this tracer for the lymphatic system and its rapid clearance from the injection sites, the potential of bringing more objective quantification to lymphoscintigraphy, which has been attempted in the past with mixed success, may be on the horizon.[45,46] [**See Figure, Supplemental Digital Content 2**, which shows retention curves of technetium 99–sulfur colloid (*left*) and tilmanocept (*right*) from the injection sites. Technetium 99–sulfur colloid retention is noted to be 90 percent versus 65 percent for tilmanocept at 6 hours (images courtesy of Dr. Kevin Donohoe, Nuclear Medicine, Beth Israel Deaconess Medical Center, Boston, Mass.; reprinted with permission. Previously published in *Lymphedema Surgical Approach and Specific Topics*, Chapter 3, Clinical Diagnosis of Lymphedema, by Bao Tran B, Singhal D, Copyright Elsevier, 2018), ***http://links.lww.com/PRS/D205***.]

Further advancements in perioperative lymphatic imaging may emerge from radiotracers with dual modalities. For example, indocyanine green–99mTc-nanocolloid is a tracer with both radioactive and near-infrared fluorescence and has been demonstrated to outperform both indocyanine green and blue dyes in intraoperative lymphatic optical detection, with considerably less injection volume and longer retention time.[47]

MAGNETIC RESONANCE IMAGING

In addition to lymphoscintigraphy and indocyanine green lymphangiography, magnetic resonance is a powerful imaging modality for the evaluation of lymphedema, with a tremendous advantage being the complete avoidance of radiation. Magnetic resonance angiography has been reported to aid in the localization of lymph nodes for transfer.[48] Moreover, magnetic resonance angiography can provide additional soft-tissue details in a patient with lymphedema, including confirming patency of vessels, defining areas and severity of soft-tissue edema, evaluating fat content, and performing volumetric measurements[49] (Fig. 2).

Magnetic resonance lymphangiography is another modality for evaluating the presence and severity of peripheral lymphedema, visualizing

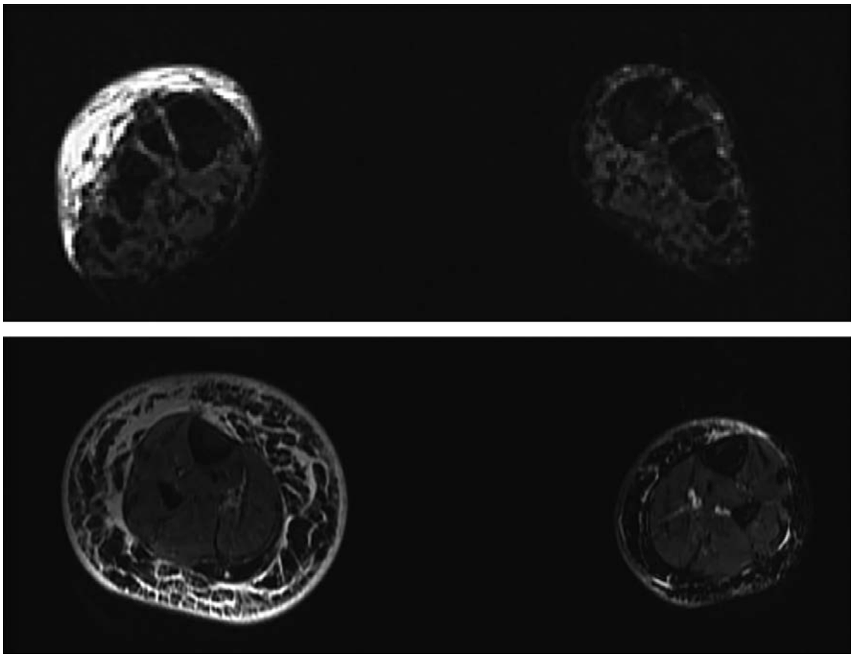

Fig. 2. Representative magnetic resonance images from an assessment of right lower extremity lymphedema, performed at 3.0 T. Axial fluid-sensitive, fat-suppressed, short-tau inversion recovery images at the midfoot (*above*) and midcalf (*below*) demonstrate marked edema of the right leg in comparison with the left, denoted by areas of high signal intensity, with marked thickening of the subcutaneous soft tissues. The muscles are spared. (Images courtesy of Dr. Leo Tsai, Body Imaging Radiology, Beth Israel Deaconess Medical Center, Boston, Mass.)

individual superficial lymphatic channels, and assessing the status of the surrounding subcutaneous tissue.[50,51] There are two primary sequence components of magnetic resonance lymphangiography, including a three-dimensional heavily T2-weighted sequence to depict the severity and extent of the lymphedema and a fat-suppressed, three-dimensional, spoiled gradient-echo sequence performed after the intradermal injection of an extracellular gadolinium-based magnetic resonance contrast agent to visualize enhancing lymphatic channels and sites of dermal backflow.[51] This imaging modality is particularly important for surgical planning, as it helps identify lymphatic channels and targeted lymph nodes for lymphovenous bypass and vascularized lymph node transfer, respectively.[48,51] In addition, magnetic resonance lymphangiography offers the possibility of postprocessed three-dimensional reconstruction of structures preoperatively.[51] Venous contamination, or difficulty differentiating between lymphatic and venous structure, is a common challenge with magnetic resonance lymphangiography. Solutions to this problem are either a delayed magnetic resonance venogram or intravenous administration of an iron-oxide blood-pool magnetic resonance contrast agent to remove the venous signal.[51]

COMPUTED TOMOGRAPHY

Although the superiority of magnetic resonance imaging is well established in characterizing soft tissues in comparison with computed tomography, two modalities of computed tomography deserve mention. Single-photon emission computed tomography is a hybrid imaging modality that yields morphologic information on computed tomography and functional information on single-photon emission computed tomography and positron emission tomography.[52–54] Serial two-dimensional images of radioactive tracers are captured with a specialized gamma camera that is rotated around the patient every 15 to 20 seconds. A computer is then used to apply a tomographic reconstruction algorithm to the multiple projections, yielding a high-resolution three-dimensional data set.[52] The three-dimensional depiction and superior spatial resolution of single-photon emission computed tomography allow for the precise anatomical identification of lymphatic

Table 2. Intraoperative Lymphatic Imaging Technologies

Technology	Contrast	Penetration Depth	Radiation	Depth Resolved	Acquisition Time	Notes
SPECT-CT	Radioactive tracer	Whole body	Yes	Yes	20–60 min	Three-dimensional resolution; highest radiation exposure; highest detection sensitivity; best for preoperative investigation
Fh-SPECT	Radioactive tracer	4–5 cm	Yes	Yes	~10 min	Handheld; three-dimensional visualization within operating room; best for intraoperative guidance
Hybrid gamma imaging	Radioactive tracer	4–5 cm	Yes	No	1–4 min	Handheld; high sensitivity; low cost; best for intraoperative guidance
Immunofluorescence	Fluorescence bound antibody	2 cm	None	No	10–1000 msec	Low-cost; nonionizing; dynamic; best for intraoperative guidance or superficial investigation
Near-infrared fluorescence	Fluorophore	2 or 4 cm*	None	No	10–1000 msec	Low-cost; nonionizing; fast; modest depth penetration; best for intraoperative guidance or superficial investigation

SPECT-CT, single-photon emission computed tomography; Fh-SPECT, freehand single-photon emission computed tomography.

abnormality, assessment of surrounding connective tissue, and visualization of dermal backflow.[52] Such information is critical for microsurgical planning, particularly in finding connection capabilities for microsurgical lymph vessel transplantation and lymphovenous anastomosis.[52]

Another modality, computed tomography–lymphography, offers three-dimensional, high-resolution imaging of the lymphatic system.[55–58] Compared with lymphoscintigraphy, computed tomography–lymphography can visualize individual lymphatic channels as small as 0.7 to 1.2 nm in diameter and also offers three-dimensional anatomical evaluation of deeper tissues. Radiation exposure is the main disadvantage of this imaging modality.

INTRAOPERATIVE TECHNOLOGY

Although surgical instrumentation has been enhanced to facilitate supermicrosurgical anastomoses, including high-power microscopes (50×; MM50; Mitaka Kohki Co. Ltd., Tokyo, Japan), supermicrosurgical forceps (0.06 mm; Togari; EMI Factory Co., Nagano, Japan), and needles (12-0 nylon; Crownjun Kono Co., Tokyo, Japan),[30] intraoperatively, lymphatic surgeons must first localize and isolate lymphatic channels often measuring 0.2 to 0.6 mm. Further complicating this process is that lymph fluid can be imperceptible by the naked eye, making it possible for a surgeon to completely transect a lymphatic vessel and not realize it. Intraoperative advances in lymphatic surgery will bring to light the invisible (Table 2). The most ideal method of lymphatic visualization will be those that allow for "live surgery," or the ability for the surgeon to simultaneously identify, magnify, and dissect a targeted lymphatic vessel.

DYES AND FILTERING BACKGROUND

Intraoperative lymphatic visualization is most commonly performed with near-infrared fluorescence imaging using the Photodynamic Eye system commonly used in lymphangiography.[20,59] To visualize the fluorescent signal, the lights in the operating room are often dimmed and the surgeon must look away from the operative field to detect the white signal with a black background on a separate monitor, which can be disorienting (Fig. 1). Although near-infrared technology is powerful in allowing for a 2- to 4-cm depth of penetration, the current methods of evaluation do limit the lymphatic surgeon's ability to perform live surgery.

A modified Photodynamic Eye fluorescence camera has been created to work in ambient light, thus minimizing disturbance during surgery and providing real-time guidance during lymphatic dissection. This feat is achieved by synchronizing pulsed excitation illumination and the camera for capturing the background plus the fluorescence signal and then immediately acquiring a background-only image. The two images are then subtracted to provide a real-time "pure" fluorescence image. The modified Photodynamic Eye fluorescence camera enhances fluorescence imaging properties and improves lymphatic detection sensitivity and thus will be a promising modality in fluorescence-guided surgery.[60] A slightly different approach is to use frequency gating to filter out background signal. Here, a technique called frequency-domain photon migration uses time-varying excitation that produces a time-varying fluorescence signal, effectively isolating and filtering any constant background signal.[61]

Immunofluorescence is often used to visualize lymphatic vessels that are not visible with conventional microscopy. Immunofluorescence has the advantage of using peptides to target lymphatic vasculature of interest, such as tumor cells,[62] lymphatic endothelial cells,[63] and immune cell migration.[64] After local injections of dyes such as fluorescein isothiocyanate-dextran–labeled dextran (Pharmacia & Upjohn, London, United Kingdom), lymphatics can be visualized by adapting traditional surgical microscopes with fluorescence emission filters (approximately 450 to 490 nm for fluorescein isothiocyanate, and approximately 815 to 850 nm for indocyanine green).[65–69] This currently available technology has been used to perform live surgery during the lymphedema microsurgical preventative healing approach (Fig. 3).[70] [**See Video, Supplemental Digital Content 3**, which shows fluorescein isothiocyanate to visualize arm lymphatic channels during the lymphedema microsurgical preventative healing approach procedure (used with permission from Wolters Kluwer Health, Inc.), ***http://links.lww.com/PRS/D206***.] This powerful property of fluorescein isothiocyanate is also apparent in lymphovenous anastomosis in the treatment of chronic lymphedema (Fig. 4).

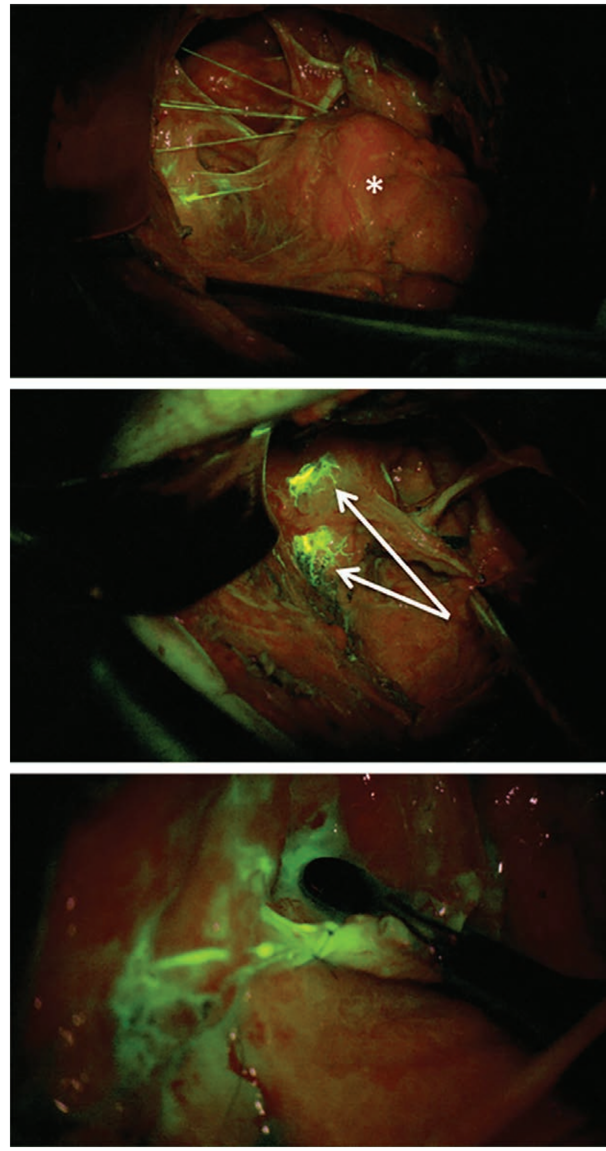

Fig. 3. Fluorescein isothiocyanate is a powerful imaging tool used during the lymphedema microsurgical preventative healing approach. Fluorescein isothiocyanate is injected intradermally in the upper inner arm before the axillary lymph node dissection. (*Above*) Before excision of level 1 lymph nodes (*asterisk*), arm lymphatics are seen emerging from the arm. (*Center*) After lymphadenectomy, the arm lymphatics are seen divided in two distinct anatomical locations (*arrows*). (*Below*) Fluorescein isothiocyanate confirms patency of the lymphovenous anastomosis.

PORTABLE GAMMA PROBE AND REVERSAL LYMPHATIC MAPPING

Vascularized lymph node transfer has become increasingly popular for chronic lymphedema treatment.[48,71–73] However, the risk of donor-site lymphedema remains a significant concern. As such, reverse lymphatic mapping, including a preoperative distal extremity technetium injection, has been proposed to identify and exclude lymph nodes that drain the donor site in the flap design. Intraoperatively, a gamma detector is used to localize nodes to be preserved during flap harvest.[74]

Although radioguided surgery with the use of gamma detectors is now well established,[75] there has been a push for developing hand-held gamma probes capable of creating a full field of view for image guidance.[76–79] Still in their early development, these imaging probes are not quite real-time (1 to 4 minutes), but can probe centimeters of tissue depth for optimized localization and guidance.[80] Testing protocols have been suggested[81] and demonstrated[80] to quantitatively compare systems for performance and utility over several metrics. Recently, a hybrid gamma camera system has been developed to simultaneously acquire gamma and near-infrared fluorescence images, enabling multiplexed sensing for potentially increased sensitivity and specificity of feature detection.[82]

Video 2. Supplemental Digital Content 3 shows fluorescein isothiocyanate to visualize arm lymphatic channels during the lymphedema microsurgical preventative healing approach procedure. (Used with permission from Wolters Kluwer Health, Inc.), ***http://links.lww.com/PRS/D206***.

HYBRID FREEHAND SINGLE-PHOTON EMISSION COMPUTED TOMOGRAPHY AND ULTRASOUND

The freehand single-photon emission computed tomography technology was developed by a joint research group of the Faculty of Computer Science and the Faculty of Medicine at Technische Universität München and uses three-dimensional images, thus providing additional depth information and the possibility of image fusion with a live video of the operation situs.[83] Before incision, a region of interest is scanned for radioactivity by means of a hand-held gamma probe using freehand single-photon emission computed tomography (DeclipseSPECT; SurgicEye, Munich, Germany). Three-dimensional reconstruction of imaging data is then projected onto the surface of the patient using a screen display (Fig. 3).[84] Freehand single-photon emission computed tomography has been shown to be more sensitive than conventional acoustic gamma probe detection for intraoperative identification of sentinel lymph nodes in breast cancer.[84] In particular, the three-dimensional depth information is particularly helpful for localizing lymph nodes deep in the axilla or in obese patients.

A multimodal freehand single-photon emission computed tomography and ultrasound system has been developed by Matthies et al. to provide additional morphologic information during surgery.[85] In this system, freehand single-photon emission computed tomography acquires a three-dimensional radioactivity distribution data set for a region of interest that is then used to overlay real-time B-mode images from the commercial ultrasound system.[86] This overlay is made possible by an infrared tracking system (Polaris Vicra; NDI, Waterloo, Ontario, Canada) that has been incorporated into the freehand single-photon emission computed tomography camera head. Real-time fusion of freehand single-photon emission computed tomography and ultrasound has a sentinel lymph node co-registration rate of approximately 75 percent, although this is much lower for patients with multiple lymph nodes next to the radioactive hotspot or if the nodes are too deep.[87] Compared to single-photon emission computed tomography, this technology has lower exposure time (10 minutes versus 20 minutes) and no radiation exposure from computed tomography.[87,88]

FUTURE DIRECTIONS

The diversity of ongoing technological innovations in perioperative imaging including intraoperative dyes and cameras offers an unprecedented opportunity for the field of lymphatic surgery to continue flourishing and evolve. As additional advances emerge, we need a systematic and objective way of evaluating the efficacy and clinical integration readiness of such technologies. Without question, these technologies will help lymphatic surgery trend toward increasing objectivity, which will be critical for continued evolution and advancement.

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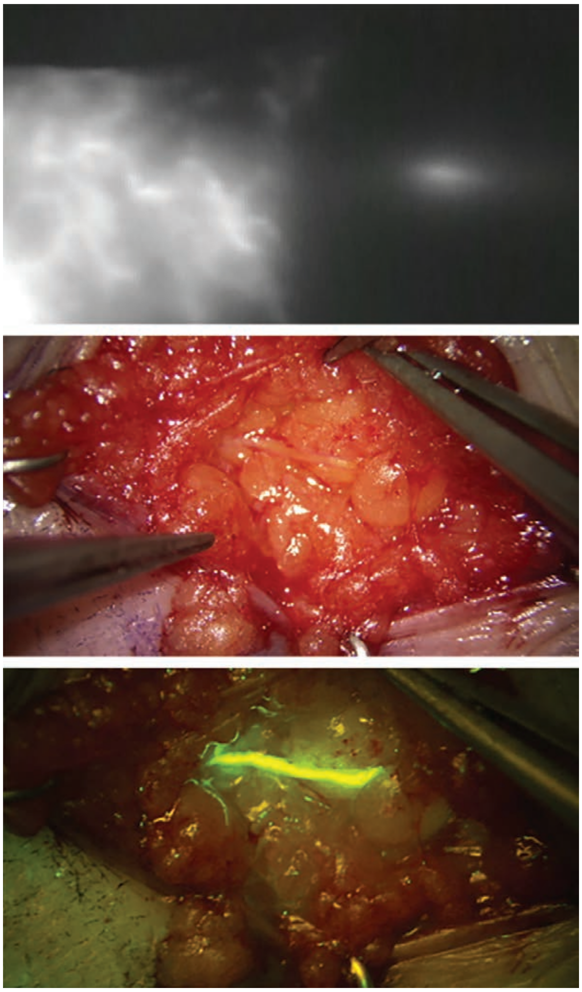

Fig. 4. (*Above*) Preoperative indocyanine green lymphangiography, providing a 2-cm depth of penetration, demonstrates a single dominant lymphatic channel in the proximal upper arm with a diffuse pattern noted distally. (*Center* and *below*) Intraoperatively, the lymphatic channel is localized using a Mitaka MM51 microscope equipped with the FL-Y package. The yellow filter is activated (*below*) and deactivated (*center*) with a foot pedal, allowing simultaneous visualization, magnification, and dissection. Two images (*center* and *right*) are as visualized through the binoculars of the microscope.

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