

The FLARE™ Intraoperative Near-Infrared Fluorescence Imaging System: A First-in-Human Clinical Trial in Breast Cancer Sentinel Lymph Node Mapping

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ABSTRACT

Background. Invisible NIR uorescent light can provide high sensitivity, high-resolution, and real-time image-guidance during oncologic surgery, but imaging systems that are presently available do not display this invisibility in the context of surgical anatomy. The FLARE™ imaging system overcomes this major obstacle.

Methods. Color video was acquired simultaneously, and in real-time, along with two independent channels of NIR uorescence. Grayscale NIR uorescence images were converted to visible “pseudo-colors” and overlaid onto the color video image. Yorkshire pigs weighing 35 kg (n=5) were used for nal preclinical validation of the imaging (FOV), and x-ray uoroscopy exposes patients and caregivers to ionizing radiation. Importantly, neither technique is amenable to targeted contrast agents, which are a requirement for many procedures, such as image-guidance oncologic surgery (reviewed by Frangioni¹). Near-infrared (NIR) light, in the wavelength range of 700 to 900 nm, offers several significant advantages over presently available imaging techniques, including relatively high photon penetration into, and out of, living tissue, due to reduced absorbance and scatter, and a relatively high signal-to-background ratio (SBR) due to low tissue autofluorescence (reviewed by Frangioni¹). However, NIR light is invisible to the human eye, so special optical imaging systems are required to “see” it on the surgical field. A NIR uorophore is a chemical compound that converts NIR light of one wavelength into NIR light of a different wavelength. By shining high-intensity, invisible NIR light of one wavelength onto the surgical field, and detecting invisible NIR light of this different wavelength, the exact location of the NIR uorophore can be determined. By targeting the NIR

Results. The FLARE™ system permitted facile positioning in the operating room. NIR light did not change the look of the surgical field. Simultaneous pan-lymphatic and SLN mapping was demonstrated in swine using clinically available NIR uorophores and the dual NIR capabilities of the system. In the pilot clinical trial, a total of nine SLNs were

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uorophore to a particular lumen, tissue, or organ, invisible FLARE™ Imaging System Components

NIR uorescent light can be used to “see” any desired

structure, or structures, on the surgical eld. NIR uorescent The portable cart and articulating arm were from Yan- light has already been used clinically by several groups fokee Modern Engineering (Groton, MA). The satellite sentinel lymph node (SLN) mapping in breast cancer, gastrimonitor stand was from GCX (Petaluma, CA). Ultra-sharp cancer, and colon cancer by using indocyanine green (ICG)VI monitors (20”) were from Dell (Round Rock, TX).

as the lymphatic tracer.³⁻⁸ None of these studies, however, The imaging head, comprised of a high-power, computer- was able to visualize invisible NIR uorescence in the controlled LED light housing, custom optics, custom I- context of surgical anatomy, or used an ICG formulationration, and cameras, has been described in detail optimized for clinical use.

In 2002 our laboratory introduced the rst prototype of a tem.^{11,13} Hardware was engineered to meet all relevant surgical imaging system that could acquire color video and subsections of the Association for the Advancement of NIR uorescence emission simultaneously and in real-Medical Instrumentation (AAMI)/International Electro- time.⁹ The features of this system included acquisition oftechnical Commission (IEC) standard #60601. A

NIR uorescence emission in the context of surgicalcustomized software system using the NIH-developed anatomy (i.e., provided by the color video camera) and noExtensible Imaging Platform (XIP) enabled control of the change to the look of the surgical eld, because NIR lighthardware, and permitted simultaneous acquisition of ima- is invisible. This so-called FLARE™ (Fluorescence-Assis- ges from a color video camera and two NIR uorescence

ted Resection and Exploration) surgical imaging systemcameras at up to 30 fps.⁴³ The software also permitted each underwent considerable re nements over the yearsgrayscale NIR image to be “pseudo-colored” from a pal- including compatibility with large animal surgery, the ette of more than 256 visible colors and overlaid in real- addition of hands-free operation, introduction of moretime onto the color video image. For intraoperative use, a

compact optics, development of a software system capable.118”-thick acrylic splash shield having 95% optical of live and simultaneous 30 frames-per-second (fps) multi- transmission at 800 nm was hermetically bonded to a clear camera acquisition, and most recently, introduction of theplastic drape and sterilized (Medical Technique, Inc.,

type of high-power, multi-channel, computer-controlledTucson, AZ). Using sterile technique, the shield was LED light source required for human surgery.¹⁰⁻¹³ To date, inserted into the bottom of the imaging head, and the drape the FLARE™ imaging system has been validated in moreunwrapped up, and over, the entire system. Additional than 200 rodent and 100 large animal surgeries.^{9,10,12,14-42} details can be found at www.frangionilab.org Separate,

This study describes the results from a multiyear Bio-dedicated FLARE™ systems were used for animal and engineering Partnership (BRP) grant from the Nationalhuman surgery.

Institutes of Health (NIH) designed to translate the tech-

nology to the clinic, and a NIH R21 Quick Trials in *Animal Model Systems*

Imaging grant designed for rst-in-human testing of the

FLARE™ imaging system in women undergoing SLN Animals ($n = 5$) were studied under the supervision of an approved institutional protocol. Female Yorkshire pigs (E. M. Parsons and Sons, Hadley, MA) averaged 35 kg. Pigs were induced with 4.4 mg/kg intramuscular Telazol (Fort Dodge Labs, Fort Dodge, IA), intubated, and maintained with 2% iso urane (Baxter Healthcare, Deer eld, IL). Vital signs were monitored continuously. Excitation uence rate for white light, 700 nm excitation light, and 800 nm

METHODS

Preparation of NIR Fluorophores

Methylene blue injection USP (1%; 10 mg/ml) was excitation light were 20,000 lux, 2 mW/cm² and 8 mW/ purchased from Taylor Pharmaceuticals (Buffalo Grove, cm², respectively.

IL), diluted into 10 ml of saline, and injected intravenously

as a bolus at a dose of 1 mg/kg. Indocyanine green (ICG)*First-in-Human Clinical Trial*

USP (25-mg vials) was purchased from Akorn (Decatur,

IL) and resuspended in 10 ml of supplied diluent to yield a The clinical trial was approved by the Institutional 2.5 mg/ml (3.2 mM) stock solution; 0.16 ml of this ICG Review Board (IRB) of the Beth Israel Deaconess Medical

stock solution was then transferred to a 50-ml bag of BuCenter and was performed in accordance with the ethical minate (25% human serum albumin [HSA] solution; Baxterstandards of the Helsinki Declaration of 1975. The IRB

Healthcare, Deer eld, IL) to yield 10M ICG in HSA deemed the FLARE™ imaging system a “non-signi cant risk” device. All patients gave informed consent and were

anonymized. Clinical trial participants were women undergoing SLN mapping for breast cancer. All subjects received the standard-of-care with injection, on average, c 834 μ Ci 99m Tc-sulfur colloid performed by the covering nuclear medicine physician approximately 2 hours before surgery. 99m Tc-sulfur colloid was administered as four deep peritumoral injections and four subcutaneous peritumoral injections of \approx 0.2 ml each. In the operating room, a single surgeon (Dr. Troyan) injected a total of 1.6 ml of ICG:HSA, given as four deep peritumoral injections and four subcutaneous peritumoral injections \approx 0.2 ml each. The injection site was massaged for approximately 5 minutes. Lymphatic mapping using a handheld gamma probe was performed as per standard practice except that the 40,000 lux of white (400–650 nm) light illuminating the surgical field was provided by the FLARE™ imaging system, which was positioned 18" away from the patient. Settings included 14 mW/cm² of 760 nm NIR fluorescence excitation light, a 67 msec color video camera exposure time, and a 67 to 250 msec 800 nm NIR fluorescence camera exposure time.

RESULTS

Design of the FLARE™ Imaging System

In consultation with practicing surgeons, it became apparent that a general-purpose optical imaging system for surgery must: 1) show the invisible NIR fluorescent light in the context of surgical anatomy, i.e., overlaid onto the color video image; and 2) provide two independent channels of NIR fluorescent light for procedures that, for example, require resection of one tissue (such as a tumor) as well as avoidance of other tissues (such as nerves and blood vessels). These design criteria were satisfied using the optical imaging system components shown in Fig. 1. By paying careful attention to light filtration, and by using dichroic mirrors to direct light to a color video camera or two different NIR cameras (Fig. 1b), the FLARE™ imaging head is capable of simultaneous, real-time image acquisition from all three cameras.¹³ Detailed specifications of the FLARE™ imaging system are provided in Table 1 and include an adjustable FOV from 2.2 to 15 cm, a resolution of 125 to 625 μ m, and an 18" working distance between the imaging head and the patient. A graphical user interface included with the software provides simple, point-and-click control of the system.

Deployment in the Operating Room

The FLARE™ imaging system is built on a portable cart designed to withstand up to 10° of tilt. The cart houses two

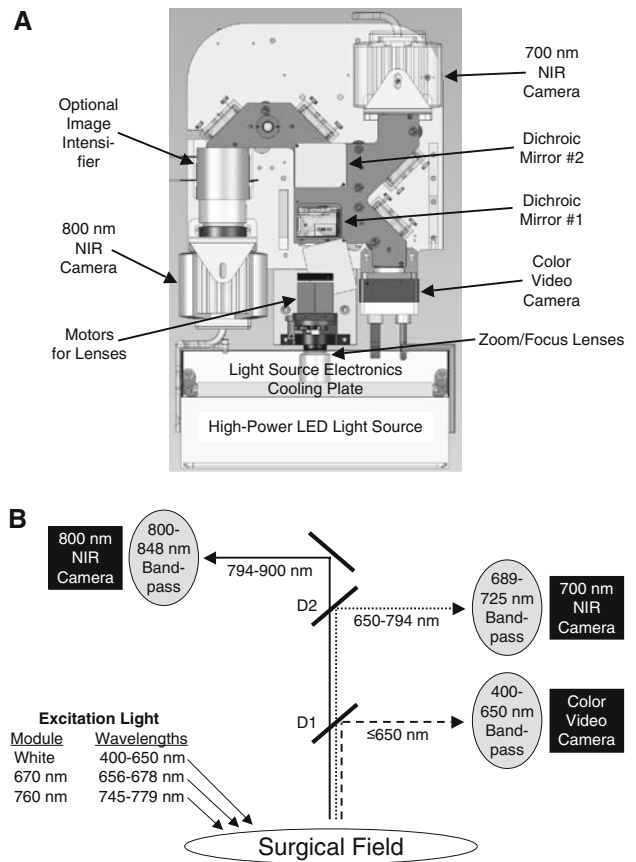


FIG. 1 FLARE™ Near-Infrared Fluorescence Imaging System. Detailed schematic of imaging head filtration for the FLARE™ imaging system having a color video camera, and two independent and simultaneous NIR cameras (700 nm emission and 800 nm emission). D1 = 680 nm dichroic mirror. D2 = 770 nm dichroic mirror

monitors for the technologist operating the system. One monitor displays control software, and the other displays a duplicate of the surgeon's monitor. The surgeon's monitor is on a satellite pole, which can be positioned in any convenient location up to 16 feet from the cart. Deployment of the system in the operating room where the clinical trial described below was conducted is shown in Fig. 2. Attached to the cart is a specially designed 6 degree-of-freedom (DOF) articulated arm. Depression of either one of the two brake release buttons on the imaging head handles releases the arm brakes and permits smooth and precise positioning of the imaging head over the surgical field (Fig. 2b). Release of the button engages the brakes and fixes the location of the imaging head in three-dimensional space. The overall reach of the articulated arm is 43–70" from the floor and up to 50.7" from cart.

Hands-free operation of the FLARE™ imaging system is accomplished using a 6-pedal foot switch (not shown), which can be configured for any software functions. For this study, the foot switch was programmed for zoom-in

TABLE 1 FLARE™ Imaging System Specifications

Category	Specification	Description
Physical	Size	Mobile cart: 32" W x 32" D x 41.4" H; Mast height: 82"
	Weight	675 lb, including all electronics
	Arm	6 degree-of-freedom; Reach: 43–70" from floor, 50.7" from cart
Electrical	Voltage and plug	120 V AC, 60 Hz; single NEMA 5-15 120 V/15 A AC plug
	Current	15 A max
	Grounding	Isolation transformer for all components; redundant chassis grounding
	Leakage current	<300 μ A (per AAMI/IEC #60601)
Sterility	Shield	Disposable acrylic shield with 95% transmission
	Drape	Disposable, custom-fit plastic drape bonded to shield
Light source	Housing	Anodized aluminum with secondary 400-W cooling plate
	Elements	Custom 25-mm circular LED arrays w/ integrated linear drivers
	Electronics	Custom passive and active boards with embedded controller
	Fluence rates	40,000 lux white light (400–650 nm), 4 mW/cm ² at 700 nm (656–678 nm) excitation light, 14 mW/cm ² at 800 nm (745–779 nm) excitation light
Optics	Working distance	18" (45 cm) from surface of patient
	Field-of-view	2.2 W x 1.7 H cm to 15 W x 11.3 cm (adjustable zoom)
	Emission/reflection Channels	Color video (400–650 nm), 700 nm fluorescence (689–725 nm), 800 nm fluorescence (800–848 nm), all with simultaneous acquisition
	Pixel resolution	640 x 480 for each camera
	System resolution	125 x 125 μ m (x,y) to 625 x 625 μ m (x,y)
	Display refresh	Up to 15 Hz simultaneous acquisition on all 3 cameras
	NIR exposure time	Adjustable from 100 sec to 8 sec
Hands-free	Optics	Automatic zoom/focus
	Control	6-pedal foot switch
Monitors	Number	2 cart-mounted 20" for operator; 1 satellite 20" on stand for surgeon

(with auto-focus), zoom-out (with auto-focus), single-shot low quantum yield (QY) and small hydrodynamic diameter image acquire, single-shot image recall, cine (movie) save (HD).³⁶ The relatively low QY results in decreased and cine (movie) recall. brightness of the NIR fluorescence signal, and the small

Imaging system sterility was achieved with a sterile HD results in ICG owing through the SLN into second-splash shield/drape combination, which could be applied in tier nodes. However, simple incubation of ICG with human the operating room using sterile technique, and which serum albumin (HSA) resulted in a 1:1 complex covered the imaging head, articulated arm, and cart. (ICG:HSA), which had a threefold higher QY, and a HD of ≈ 7 nm, which improves SLN retention.³⁶

Final Preclinical Validation

To complete preclinical validation of the FLARE™ imaging system for lymph node mapping, Yorkshire pigs are currently constrained by the fact that there are only two clinically available NIR fluorophores: methylene blue colon parenchyma with 10 μ g of ICG:HSA, prepared as (MB) and ICG⁴², neither is presently FDA-approved for described in *Materials and Methods*. In unpublished work, lymph node mapping. Fortunately, these agents fluoresce *we* have found that intravenously injected MB is excreted different NIR wavelengths, with MB having peak emission into bile but then reabsorbed by the intestine, resulting in in the 700-nm range and ICG in the 800-nm range. MB and very bright 700 nm NIR fluorescence of all mesenteric ICG thus provide the means to test the full capabilities of lymph nodes. Thus, MB can be used to visualize all lymph the FLARE™ imaging system under realistic clinical nodes of the mesentery, whereas ICG:HSA can be used to conditions. identify which node is the SLN. As shown in Fig, the

Previously, we have demonstrated that ICG alone was dual NIR capabilities of the FLARE™ imaging system nonoptimal agent for SLN mapping, due to its relatively permitted sensitive, real-time identification of all lymph

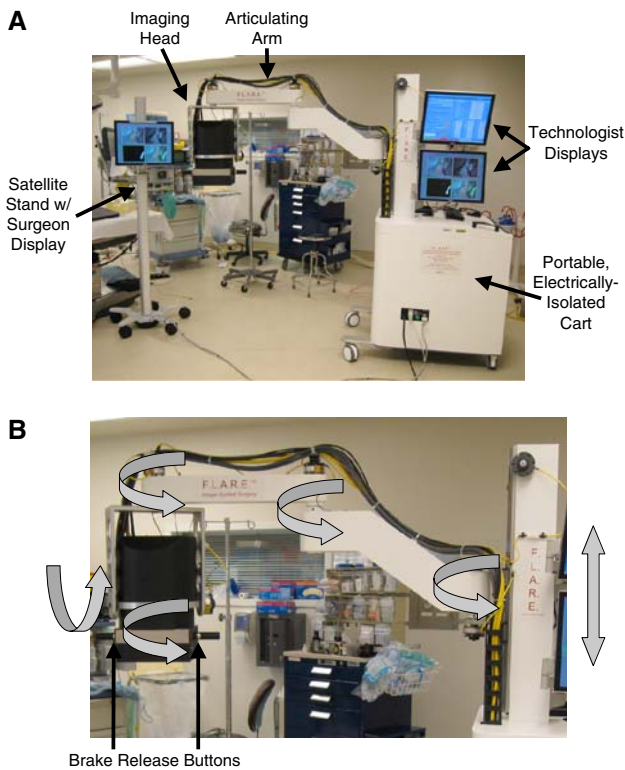


FIG. 2 Deployment in the operating room and range of motion. Portable imaging system and satellite monitor stand deployed in the operating room. Foot switch not shown. Six degrees-of-freedom range of motion (gray arrows) and brake release buttons of the imaging head

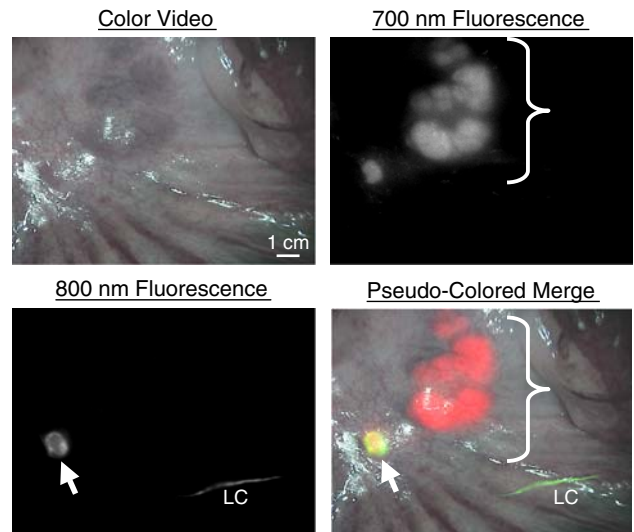


FIG. 3 Simultaneous, NIR uorescent pan-lymph node mapping and sentinel lymph node mapping in swine. All lymph nodes of the mesentery (bracket) uoresce at 700 nm (pseudo-colored red) after a single intravenous injection of 1 mg/kg of methylene blue. SLN (arrow) uorescing at 800 nm (pseudo-colored green) is identified after intraparenchymal injection of 100 μM of ICG:HSA into the colon wall, and appears yellow. The lymphatic channel (LC) feeding the SLN appears green. All camera exposure times were 67 msec. Data are representative of n = 5 pigs

nodes present in the surgical field, as well as definitive identification of the SLN. Importantly, the “pseudo-color” and overlay features of the software permitted color-coding of each type of node, with all nodes being displayed in red, the SLN and its lymphatic channels being displayed in green, and overlap of the two colors appearing as yellow (Fig. 3).

First-in-Human Clinical Trial

After IRB approval, a 6-patient clinical trial of the FLARE™ imaging system was conducted in women undergoing SLN mapping for breast cancer. All patients provided informed consent and received the standard-of-care with ^{99m}Tc-sulfur colloid lymphoscintigraphy as described in *Materials and Methods*. Subjects also received intratumoral/subcutaneous injection of ICG:HSA in the operating room, followed by massage of the site for 5 minutes. The FLARE™ imaging system was used to image the surgical field before, during, and after ICG:HSA injection. Because NIR light is invisible, there was no change to the look of the surgical field. On the monitor, however, the surgeon was able to visualize the injection

lymphatic flow, and retention of ICG:HSA and could use the color-NIR merged image to exactly pinpoint the lymph node of interest.

The clinical characteristics of all patients are provided in Table 2, and typical results from intraoperative imaging are shown in Fig. 4. Patient 1 was an interesting case, in whom three SLNs were identified using conventional ^{99m}Tc-sulfur colloid lymphoscintigraphy but four SLNs were identified using NIR uorescence (Fig. 4a). The radioactive counts of the fourth SLN, seen by NIR uorescence but not lymphoscintigraphy, were no higher than background. Importantly, this fourth lymph node had macroscopic breast cancer metastases (Table 2).

In four of six patients, the SLNs identified by lymphoscintigraphy were the same as those identified by NIR uorescence. In patient 6, two SLNs were identified by lymphoscintigraphy and only one by NIR uorescence. Overall, a total of nine SLNs in six patients were identified by ^{99m}Tc-sulfur colloid lymphoscintigraphy, and nine SLNs were identified by NIR uorescence. The real-time and high-resolution features of the FLARE™ imaging system are highlighted in Fig. 5 using the data from Patient 2. In this case, lymphatic flow deep within the axilla could be visualized and, during SLN resection, there was unambiguous discrimination of the SLN from surrounding non-SLNs.

TABLE 2 Patient characteristics, identification of SLNs, and pathology results

Patient	Age (yr)	Height (ft)	Weight (lb)	Body mass index	Skin type	Location of primary tumor	Size of primary tumor (cm)	Histological type	Histological grade	Histological tumor number	Node identified using radioactivity	Node identified using NIR uorescence	Node histology	Axillary node dissection (Pos/Tot)
1	65	5'1"	160	30.2	I	Lt; UO	1.3	ILC	II	1	Yes	Yes	-	0/6
2	62	5'7"	230	36.0	III	Rt; UO	0.9	IDC	I	1	Yes	Yes	-	n.d.
3	64	5'4"	178	30.6	II	Lt; IC	1.8	IMPC	II/III	1	Yes	Yes	m	0/11
4	51	5'4"	152	26.1	III	Rt; UC	0.7	IDC	I	1	Yes	Yes	-	n.d.
5	65	5'7"	187	29.3	III	Lt; LO	0.9	IDC	III	1	Yes	Yes	-	n.d.
6	60	5'4"	160	27.5	II	Lt; UC	1	IDC	II	1	Yes	No	-	n.d.
Totals										2	9	9	-	

Skin type = American Academy of Dermatology Skin Types I-VI:

I. Pale, white skin: always burns easily; never tans (Celtic, Scandinavian, and infants)

II. White: usually burns easily; tans minimally (Northern European)

III. White (average): sometimes burns; tans gradually to light brown (Central European)

IV. Beige or lightly tanned: burns minimally; always tans to moderately brown (Mediterranean, Asian)

V. Moderate brown or tanned: rarely burns; tans well (South American, Indian, Native American)

VI. Dark brown or black: never burns; deeply pigmented (African, African-American, Aborigine)

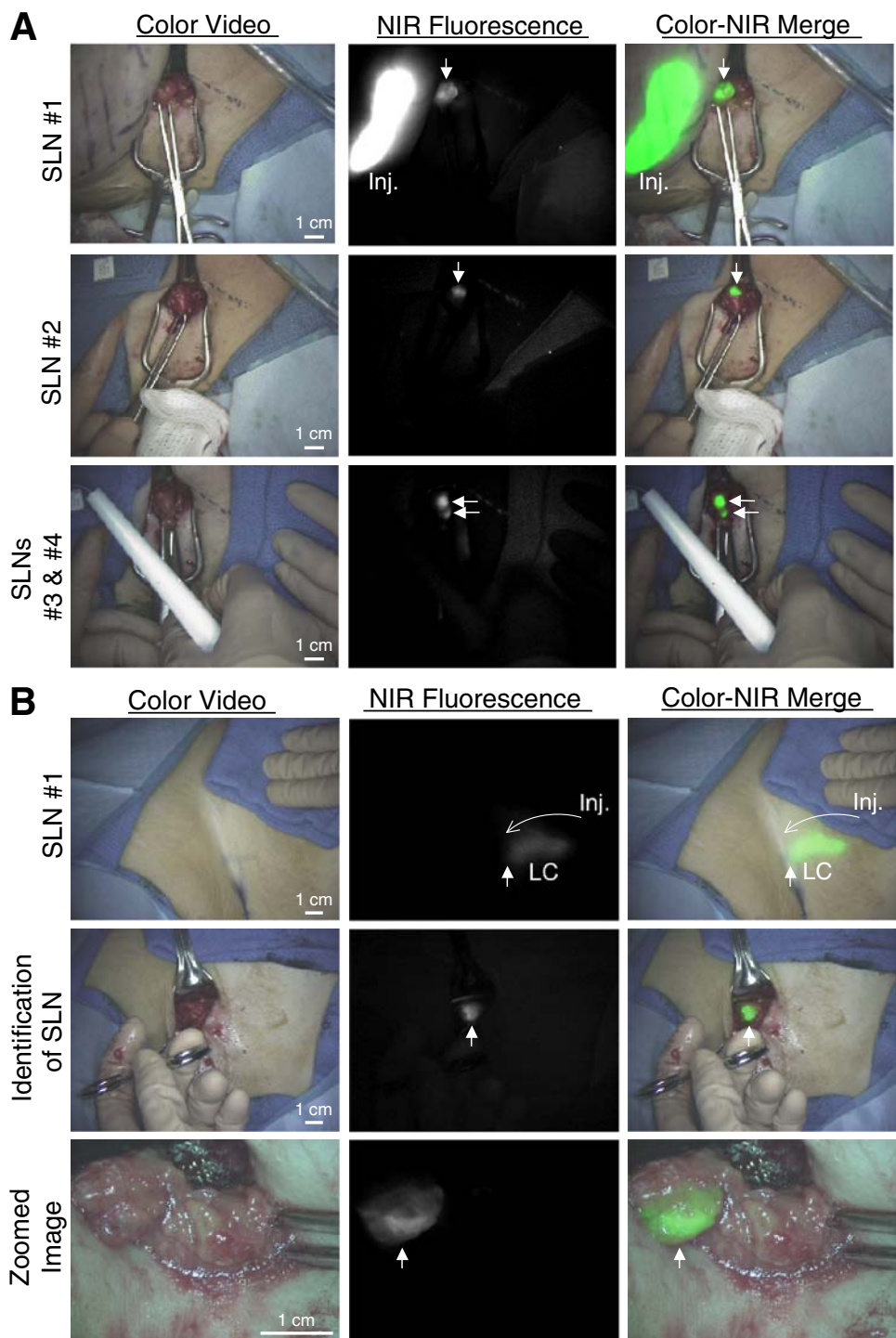
Location of primary tumor: Lt = left; Rt = right; C = center; I = inner; L = lower; O = outer; U = upper

Histological types: IDC = invasive ductal carcinoma; ILC = intralobular carcinoma; IMPC = invasive micropapillary carcinoma

Node histology:- = no evidence of metastasis; m = micrometastases; ? = metastases

Axillary node dissection: Pos = number of positive lymph nodes identified; Tot = total lymph nodes identified; n.d. = not done

FIG. 4 NIR uorescent sentinel lymph node mapping in women with breast cancer. Shown are color video images (*left*), 800 nm NIR uorescence images (*middle*), and a pseudocolored (*lime green*) merge of the two (*right*) after injection (Inj.) of 10 μM ICG:HSA. a Four SLNs (*arrows*) identi ed and resected for Patient #1. 800-nm camera exposure time was 200 msec. Single SLN identi ed and resected for Patient #2. Shown are ow through a lymphatic channel (LC) and position of the SLN (*arrow; top row*), identi cation of the SLN (*arrow; middle row*), and a zoomed image of the SLN (*arrow*) during resection (*bottom row*). Note multiple non-SLNs nearby. 800-nm camera exposure time was 200 msec



DISCUSSION

NIR uorescence-guided surgery requires optimized resolution over a large FOV. The key feature of the FLARE™ imaging system is its ability to see color video images (i.e., surgical anatomy) simultaneously with two independent channels of NIR uorescence. The importance of dual NIR capability in this system is completely portable, electrically isolated and highlighted in Fig. 3; one can imagine how these two NIR

safety, provides facile positioning over the patient, is noncontact (18" working distance), and provides micron-

channels (700 nm and 800 nm) could be exploited for any to be dim or off. The NIR excitation light for the 700 nm surgical procedure. During cancer surgery, for example, emission channel will tend to “tinge” the surgical field in a one NIR channel could be reserved for resection of the reddish hue unless it is properly balanced with white light. tumor and the other for avoidance of nerves. During vasFinally, a minimally invasive (laparoscopy) version of the cular surgery, one NIR channel could be reserved foFLARE™ imaging system will be needed for many non-detection of intravascular thrombi and the other for vas-open surgeries, and such a system is under active cular patency or anastomotic leak.^{10,41,42} During general development.

surgery, one channel could be reserved for identi cation of In this era of concern about healthcare costs, a transparent lumens, such as the bile ducts or ureters, and the other for discussion of the costs associated with FLARE™ technology the location of blood vessels or nerves.^{10,28,39,41} Many is warranted. The current version of the FLARE™ system is future applications of the FLARE™ technology, however, comprised of parts totaling approximately \$120,000 USD. will require development of contrast agents for speci c When purchased in quantity, and assembled using modern applications. manufacturing techniques, the true cost of the system will be

ICG:HSA is a reasonable, short-term solution as æ much smaller fraction of this amount. The ICG:HSA lymphatic tracer for SLN mapping using NIR uorescence, lymphatic tracer used in this study costs approximately \$109 although it is not ideal with respect to optical properties or per patient, comprised of \$73 for the ICG and \$36 for the SLN retention. It does, however, offer signi cant advan-HSA. These costs should be considered in the context of tages over small molecule lymphatic tracers, such as MB^{99m}Tc-sulfur colloid lymphoscintigraphy. A handheld and ICG, including a larger hydrodynamic diameter, gamma probe system for SLN mapping costs approximately resulting in better SLN retention, high uorescence quan-\$20,000, and a dose of ^{99m}Tc-sulfur colloid costs approxi- tum yield (i.e., brightness), and high contrast using NIR mately \$32 per patient. Because costs associated with uorescence rather than blue color. It is dif cult to pre- sulfur colloid lymphoscintigraphy have been relatively stadi- dict whether the addition of a small molecule blue dye, ble, it is probable that NIR uorescence technology could such as isosulfan blue or MB, to ^{99m}Tc-sulfur colloid become competitive as commercialization occurs and costs lymphoscintigraphy would have improved detection of fall. Indeed, our laboratory has already developed the lymph node #4 from Patient 1, because the injection “Mini-FLARE™” version of the system (manuscript in technique (discussed below) was an uncontrolled variable preparation), which reduces costs to be competitive with in our study.⁴⁴ Fortunately, contrast agent development is conventional lymphoscintigraphy. The tangible advantages progressing rapidly in both academia and industry, and it is of NIR uorescence also must be considered. NIR uores- expected that optimal NIR uorescent lymphatic tracers for cence provides true two-dimensional imaging rather than SLN mapping will soon be available. point-probing, is real-time, helps remove ambiguity asso-

The present incarnation of the FLARE™ imaging system ciated with SLN detection, could ultimately save is not without limitations. The surgeon conducting this considerable operating room time, could eliminate the need study preferred the imaging head to be 90° relative to the for both a nuclear medicine physician and injection of ion- oor, facing directly into the axilla. Such an angle would izing radiation, and guides the pathologist to precise location be better served by a smaller, more compact imaging head within the node (i.e., sinus entry site) that is most likely to Although NIR uorescence excitation uence rate (i.e., harbor metastases.

brightness) was adequate to pinpoint SLNs that were sev- The rst-in-human trial of the system con rmed several eral centimeters deep in the axilla, a higher uence rate observations made during previously published rodent and would have permitted shorter NIR camera acquisitions wine lymphatic mapping studies.^{12,16,18,19,25,27,31–34,36,40}

times. Previously, we have dened the photobleaching First, NIR light did not change the look of the surgical threshold for NIR uorophores of the heptamethine indo- eld, so the learning curve for using the technology was cyanine class.^{18,23} In general, uence rate should be kept to minimal. Second, NIR uorescence could be used to see

B50 mW/cm², which means that the present FLARE™ lymphatic ow and to pinpoint the general location of the imaging system, having a 14 mW/cm² uence rate for SLN even before an incision is made. The body habitus of 760 nm excitation light, could be ≈3.5-fold brighter the six patients differed over a wide range, resulting in without risk of photobleaching. Marks drawn on the skin SLNs as deep as 4 cm from the surface, and yet SLN with many brands of surgical markers were NIR uores- identi cation was still possible. Skin types in these trial cent. This interfered with the ability to see draining subjects only ranged from types I to III. Darker skin is not

lymphatics. Because conventional operating room lights expected to cause signi cant problems, because pigmen- produce signi cant amounts of NIR light, white light tation is restricted to such a thin layer (1 mm); however, illuminating the surgical eld must be provided by the this must be tested formally in subjects with type VI skin. FLARE™ system, and all other operating room lights need Finally, NIR uorescence was able to unambiguously

identify SLNs, even when part of a larger cluster of surrounding lymph nodes. royalties if a product is commercialized. No other authors have any financial interest in this study.

We caution over-interpreting the SLN identification results from this pilot trial. On one hand, the sensitivity of NIR uorescence detection, using radioactive detection as the “gold standard,” was 100% (9 SLNs identified by both techniques). However, the two lymphatic tracers, ^{99m}Tc -sulfur colloid and ICG:HSA, were injected by different clinicians 2 hours apart, with no control over injection location or depth. Thus, it is possible that different lymph node territories were being interrogated. This asynchrony in time was necessary because ^{99m}Tc -sulfur colloid has a large HD and requires a long migration time to reach the SLN. On the contrary, ICG:HSA requires only 5 minutes to reach the SLN. The NIR uorescence signal in the SLN is lower at 30 minutes, and because the HD of ICG:HSA is only intermediate (~7 nm), it is not advisable to wait longer than 30 minutes to complete the procedure. In future studies, the same clinician will perform radioactive and NIR uorescent lymphatic tracer injections, which may help reduce variability in the number of SLNs identified. Of note, we saw no evidence of second-tier lymph nodes being labeled by ICG:HSA in the 5 to 30 minutes in which the mapping procedure was completed.

In summary, the FLARE™ image-guided surgery system has been successfully translated from preclinical studies to clinical studies and is now poised for further evaluation in a variety of human surgeries.

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