Published in final edited form as:

Plast Reconstr Surg. 2010 November ; 126(5): 1472–1481. doi:10.1097/PRS.0b013e3181f059c7.

The FLARE™ Intraoperative Near-Infrared Fluorescence Imaging System: A First-in-Human Clinical Trial in Perforator Flap Breast Reconstruction

Bernard T. Lee, M.D.¹, Merlijn Hutteman, M.Sc.^{3,5}, Sylvain Gioux, Ph.D.³, Alan Stockdale, M.Ed.³, Samuel J. Lin, M.D.¹, Long H. Ngo, Ph.D.², and John V. Frangioni, M.D., Ph.D.^{3,4,†}

¹Division of Plastic and Reconstructive Surgery, Department of Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA ²Division of General Medicine and Primary Care, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA ³Division of Hematology/Oncology, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA ⁴Department of Radiology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA ⁵Department of Surgery, Leiden University Medical Center, Leiden, The Netherlands

Abstract

Background—The ability to determine flap perfusion in reconstructive surgery is still primarily based on clinical examination. In this study, we demonstrate the use of an intraoperative, near infrared (NIR) fluorescence imaging system for evaluation of perforator location and flap perfusion.

Methods—Indocyanine green (ICG) was injected intravenously in six breast cancer patients undergoing a deep inferior epigastric perforator (DIEP) flap breast reconstruction after mastectomy. Three dose levels of ICG were assessed using the Fluorescence-Assisted Resection and Exploration (FLARE™) imaging system. This system uses light emitting diodes (LED) for fluorescence excitation; different from current commercially available systems. In this pilot study, the operating surgeons were blinded to the imaging results.

Results—Use of the FLARE™ system was successful in all six study subjects with no complications or sequelae. Among the three dose levels, 4 mg per injection resulted in the highest observed contrast-to-background ratio (CBR), signal-to-background ratio, and signal-to-noise ratio. However, due to small sample size, we did not have sufficient power to detect statistical significance.

[†]**Corresponding Author:** John V. Frangioni, M.D., Ph.D., BIDMC, Room SL-B05, 330 Brookline Avenue, Boston, MA 02215, 617-667-0692 Fax: 617-667-0981, jfrangio@bidmc.harvard.edu.

Author Statements of Financial Interest:

Bernard T. Lee, M.D.: None

Merlijn Hutteman, M.Sc.: None.

Sylvain Gioux, Ph.D.: None.

Alan Stockdale, M.Ed.: None.

Samuel J. Lin, M.D.: None.

Long H. Ngo, Ph.D.: None

John V. Frangioni, M.D., Ph.D.: All intellectual property for the intraoperative near-infrared fluorescence imaging system is owned by Dr. Frangioni's employer, the Beth Israel Deaconess Medical Center (BIDMC), a teaching hospital of Harvard Medical School. None of the technology is licensed at present. As inventor of the technology, Dr. Frangioni may someday receive royalties if a product is developed. Dr. Frangioni has no real or deferred equity interests, whatsoever, in this, or any other technology.

Financial Disclosures

Devices and Drugs Used in This Study: Custom-built intraoperative near-infrared fluorescence imaging system with simultaneous color video and near-infrared fluorescence capabilities (FLARE™ imaging system); Indocyanine Green (Cardiogreen, Sigma-Aldrich, St. Louis, MO/IC-GREEN™, Akorn, Decatur, IL).

for these pairwise comparisons at the multiple-comparison adjusted type-I error of 0.017. Six mg per injection provided a similar CBR, but also a higher residual background signal.

Conclusions—Based on this pilot study, we conclude that NIR assessment of perforator flap breast reconstruction is feasible with an LED based system, and that a dose of 4 mg of ICG per injection yields the best observed CBR compared to a dose of 2 or 6 mg for assessment of flap perfusion.

Keywords

Perforator flap; near-infrared fluorescence angiography; indocyanine green

INTRODUCTION

The use of imaging as an adjunct is becoming increasingly popular in perforator flap reconstruction. As perforating vessels demonstrate a high degree of variability in size and location, identification of target vessels can decrease operative time and increase reliability. The currently used techniques for imaging include duplex ultrasound, CT, and MRI.¹⁻⁸ With improvements in technology and resolution, reconstructive surgeons are able to visualize small perforating vessels.

The current imaging modalities, however, rely on a static preoperative assessment. Ideally, the use of an intraoperative imaging adjunct would be most beneficial in order to provide a dynamic assessment. Changes in flap physiology and microsurgical flap transfer could be assessed more accurately if imaging was performed during surgery.

Our laboratory has previously described the use of a real time, near-infrared (NIR) Fluorescence-Assisted Resection and Exploration (FLARETM) imaging system in large animal surgery.⁹⁻¹¹ This system uses light-emitting diodes (LEDs) for fluorescence excitation and requires an intravenous injection of indocyanine green (ICG), an FDA-approved fluorophore. Because NIR light is invisible, the surgical field is unaltered. Our current system simultaneously displays real-time color video, up to two NIR fluorescence images, and merged images of all. Rapid recall of images is also available as needed.

We have described previously the ability of NIR imaging to identify suitable perforators during large animal surgery.¹² In addition, we validated the number of perforators identified with conventional x-ray angiography.¹³ The identification of perforators can be performed reliably at multiple anatomic sites, including abdominal and submental flaps.¹⁴ Finally, we were able to describe quantitative metrics to assess arterial and venous compromise.¹⁵

During an extensive pre-clinical laboratory experience with NIR fluorescence angiography using the FLARETM imaging system, we have demonstrated safety in use in over 200 rodent and 100 large animal surgeries. This study describes the successful clinical translation of this technology for use in a six-subject pilot trial in patients undergoing microsurgical perforator flap breast reconstruction with a deep inferior epigastric perforator (DIEP) flap. In addition, determination of ideal ICG dosage was performed based on a quantitative assessment.

MATERIALS AND METHODS

Preparation of NIR Fluorophore

Indocyanine green (ICG) USP (25-mg vials) was purchased from Akorn (Decatur, IL) and resuspended in 10 mL of supplied diluent to yield a 2.5 mg/mL (3.2 mM) stock solution. For each participant, four syringes were loaded with either 0.8 mL, 1.6 mL, or 2.4 mL of the ICG stock solution, equivalent to 2 mg, 4 mg, or 6 mg per injection.

FLARE™ Intraoperative Imaging System

The FLARE™ imaging system and its use in a clinical setting have been described previously for sentinel lymph node resection.⁹ Briefly, the imaging system consists of an imaging head mounted on an articulated arm and a cart containing control equipment, computer, and monitors (Figure 1). The imaging head has a 43” to 70” reach relative to the floor and a 50.7” reach from the cart, and can be positioned anywhere in 3-D space with six degrees of freedom. The system is engineered to meet all relevant subsections of the Association for the Advancement of Medical Instrumentation (AAMI)/International Electrotechnical Commission (IEC) standard #60601. A customized software system enables the real-time display of color video and two NIR fluorescence channels at up to 15 frames-per-sec. The software is capable of displaying the NIR fluorescence signal as a pseudo-colored overlay on the color video, thereby providing anatomical guidance to the surgeon. For intraoperative use, the entire system is wrapped in a sterile shield and drape (Medical Technique Inc., Tucson, AZ). Additional details can be found in ¹⁰ and at www.frangionilab.org.

Clinical Trial during Breast Reconstruction Surgery

The clinical trial was approved by the Institutional Review Board (IRB) of the Beth Israel Deaconess Medical Center and performed in concordance with the ethical standards of the Helsinki Declaration of 1975. The IRB deemed the FLARE™ imaging system a “non-significant risk” device. All subjects gave written informed consent and identifying information was anonymized. Clinical trial participants were women undergoing unilateral mastectomy and reconstruction with a microsurgical deep inferior epigastric perforator (DIEP) flap. Subjects received four injections of either 2 mg, 4 mg, or 6 mg ICG each. Two patients received each dose level. Before flap elevation, the camera was positioned 18” over one side of the abdomen and NIR imaging was performed using the FLARE™ system after an intravenous injection of ICG. Imaging system settings included 14 mW/cm² of 760-nm NIR fluorescence excitation light and a 67-msec camera exposure time. After ICG was cleared from the body and fluorescence levels restored to pre-injection levels, the contralateral side of the abdomen was imaged in an identical fashion. In this feasibility and dose-finding study, the operating surgeons were blinded to the FLARE™ images, thereby not changing the standard-of-care that patients were receiving during surgery.

After dissection of the vessels through the intramuscular course and isolation of the selected perforator vessels and vascular pedicle, the flap was imaged at the abdomen with a third ICG injection prior to transfer. The flap was then transferred to the chest and a microsurgical anastomosis was performed of the deep inferior epigastric artery and vein to the internal mammary vessels. A final assessment was performed with a fourth ICG injection. Subjects were evaluated for complications at one week and six weeks after surgery as part of regular postoperative follow-up.

Analysis of Near-Infrared Fluorescence Data

After each injection, NIR fluorescence data were acquired using the FLARE™ system continuously preceding the vascular signal; acquisition continued until two minutes after onset of vascular fill. Contrast-to-background ratio (CBR) was defined as the mean fluorescence intensity of the region of interest (ROI) minus the mean fluorescence intensity of a background ROI on the flap, divided by the mean camera noise. Signal-to-background ratio (SBR) was defined as the mean fluorescence intensity of the ROI, divided by the mean fluorescent intensity of a background ROI on the flap. Signal-to-noise ratio (SNR) was defined as the mean fluorescence signal of the ROI divided by the mean camera noise.

CBR, SBR, and SNR were calculated and recorded for each injection and compared among the three dose groups. Since this is a repeated-measures analysis with each subject receiving

multiple injections, we used a modeling method to account for within-subject measurements correlation. We first examined the distribution of CBR, SBR, and SNR via the use of the Shapiro-Wilks test to check for normality of each variable. The assumption of normality was satisfied for CBR, and SNR, but not for SBR. The variability was also heterogeneous with the standard deviation for dose 4 mg and 6 mg higher than that of dose 2 mg. Thus the modeling would also need to take into account the difference in dose-specific variability of these three variables. For CBR and SNR, with the normality assumption satisfied, we made use of the linear mixed-effects model,^{16,17} which allows the modeling of the variance-covariance matrix of the within-subject measurements. We used the compound symmetry structure to model the within- and between-subject variance components, which was assumed to be heterogeneous among the three different dose levels (the variances for dose 4 mg and 6 mg were estimated higher than that of the dose 2 mg, and these variances were used in the linear mixed-effects model). For SBR, due to the non-normality of the distribution, we used a generalized estimating equation (GEE),¹⁸ which could model the within-subject correlation via the exchangeable working correlation structure. We used linear contrasts to obtain the pairwise comparisons among the three dose levels. We set the adjusted level of significance (type-I error) to 0.017 (0.05 divided by 3 comparisons) when we made inference on the pairwise comparisons. We used the SAS/STAT (SAS/STAT Software, Version 8, SAS Institute, Cary NC) procedure MIXED and GENMOD for our statistical modeling.

RESULTS

Deployment of the FLARE™ System in the Operating Room

The preparation and ergonomics of the FLARE™ system in the operating room are similar to our previous description.⁹ The system was draped in a sterile fashion using a shield/drape combination that could be applied by a single person (scrub nurse). After draping, the imaging head enters the sterile field and is positioned at a fixed distance and position before each injection. Including positioning of the system, each injection and subsequent FLARE™ measurement required less than three minutes, thereby not significantly affecting the normal operative course.

The FLARE™ system is housed in a portable cart for easy transfer into the operating room. The cart houses two monitors for the technologist operating the system; one monitor displays the control software while the other displays a duplicate of the surgeon's monitor. The surgeon's monitor is on a satellite pole that can be positioned up to 16 feet away from the cart. The articulating head is specifically designed with six degree-of-freedom movements. Depression of a brake release button on the handle permits smooth and precise positioning over the field. Release of the button engages the brakes, thereby fixing the head in three-dimensional space. The maximum reach of the articulating head is 50.7 inches from the cart.

Intraoperative NIR Imaging of Perforator Vessels during Reconstructive Surgery

After IRB approval, six subjects participated in a first-in-human clinical trial using the FLARE™ system in reconstructive breast surgery after mastectomy. Precautions were taken to maintain the standard-of-care by blinding the surgeons to the results of NIR imaging. Subjects were assigned to three dose groups (n = 2 subjects per dose group) of 2 mg, 4 mg, or 6 mg per injection. Clinical study subject characteristics are displayed in Table 1.

Subjects received an intravenous bolus injection of ICG that was administered by the anesthesiologist at four separate, fixed moments during surgery, as described above. Representative FLARE™ images of each dose group are shown in Figure 2. CBR, SBR, and SNR were quantified using the ROI as displayed in Figure 1 and are summarized in Table 2 and Figure 3. Observed mean and median values of all three variables were higher in the 4 mg

group when compared to the 2 mg and 6 mg group. The variability of all three variables was also higher in the 4 mg and 6 mg dose. For CBR, the only comparison statistically significant at the multiple-comparison adjusted type-I error of 0.017 was between 2 mg and 6 mg (p -value: 0.0007) (see footnote Table 2). No statistically significant differences at the level of 0.017 were found for SBR and SNR among the 3 dose levels.

The average time intervals between injections 1 and 2, 2 and 3, and 3 and 4 were 19 min/36 sec, 3 h/16 min/41 sec, and 2 h/12 min/42 sec, respectively. The effect of dose on mean background fluorescence intensity between injections 1 and 2 was significant (ANOVA, $F(2, 20) = 5.761$, $p = .011$). Notably, the background fluorescence intensity was higher in the 6-mg group, when compared to both the 2-mg ($p = .009$) and 4-mg groups ($p = .008$), indicating residual ICG fluorescence.

In two cases, the surgeon selected and dissected perforator vessels that, by NIR imaging, were not the dominant vessels (example in Figure 3). CBR was measured for the NIR-dominant perforator vessel and the selected perforator vessel (Figure 4A) after all injections. As is displayed, the CBR of the previously NIR-dominant perforator vessel decreases after ligation and the CBR of the selected perforator vessel increases. These findings are consistent with the visual display seen during NIR imaging (Figure 4B).

Postoperative Complications

None of the subjects developed any adverse reactions to the injected ICG. Minor complications were reported in three cases: a small area of fat necrosis, a breast seroma, and a small area of mastectomy skin loss. All complications were within normal range and resolved uneventfully. No partial or total flap loss occurred.

DISCUSSION

This study demonstrates the successful translation of NIR angiography with the FLARE™ imaging system for use in microsurgical perforator-flap breast reconstruction. The design of our optical imaging system incorporated the ergonomic needs for use by surgeons in the operating room environment. The system is portable and completely self-contained; it can easily be transferred into the operating room and deployed rapidly. The ability to visualize the operative field simultaneously with NIR fluorescence is particularly advantageous. As the reconstructive surgeon is able to recall stored video and images, the findings can be correlated with surgical findings without any distortion.

NIR fluorescence imaging has the potential to find widespread use in plastic and reconstructive surgery, as well as general and oncologic surgery. It provides real-time guidance for tumor resection,¹⁹ rapid identification of sentinel lymph nodes,⁹ real-time avoidance of critical structures, such as nerves and blood vessels,²⁰ and quantitation of tissue metrics.¹⁵

By altering the intravenous dose of the fluorophore ICG we determined that 4 mg of ICG per injection had the highest observed CBR for clinical NIR angiography, although a larger study with more statistical power will be needed to better define the optimal dose. In addition, at a time interval of ≈ 20 min between injections, 4 mg resulted in significantly less residual background fluorescence intensity compared to 6 mg. Use of a higher dose would require a longer time interval, i.e., a longer clearance period, between doses to avoid dose stacking. It should be noted that even with repeat dosing at 6 mg, the total dose (24 mg) was below the standard package size (25 mg).

The use of a real-time, intraoperative imaging system is particularly advantageous in microsurgical perforator flap reconstruction in order to provide a dynamic assessment of flap

perfusion. In this study, we performed four assessments: both sides of the abdominal flap prior to elevation, the selected abdominal flap after isolation on the perforators prior to transfer, and the same flap after microsurgery. This permitted a comparison of perforator vessel selection and identification, analysis of flap perfusion after isolation on the perforating vessels, and differences after flap harvest and microsurgical transfer. Flap physiology changes with vessel isolation, harvest, and transfer, so that obtaining real-time NIR imaging at multiple points becomes a dynamic process. This is in contrast to preoperative imaging modalities such as duplex ultrasound, CT, or MRI, where identification of the dominant perforator is a static one-time assessment. In the future, we plan to use NIR imaging to assist directly in vessel selection and isolation, as well as to apply quantitative metrics previously validated in large animal model systems,¹⁵ to determine arterial or venous insufficiency.

In two cases, the dominant perforator identified by NIR imaging was not the perforator selected by the operating surgeon. This occurred as the surgeon was blinded to the results of the FLARE™ system. Of note, the CBR at the dominant perforator decreased after ligation, and the CBR at the selected perforator increased to the previous level of the dominant perforator (Figure 3A). This further demonstrates that flap physiology is often altered during surgery and the results will differ from preoperative imaging. The changes in flap physiology from perforator vessel isolation can only be seen with a real-time, intraoperative system.

The early clinical use of NIR imaging has been described previously.²¹⁻²⁹ The imaging systems used in these studies were limited to a handheld camcorder device. These systems provide subjective, qualitative results with low resolution. In addition, the outputs from previous generation devices were single grayscale images. As these systems used laser-induced fluorescence, the ICG dose was also higher at 0.5 mg/kg,²⁹ so that an average 70-kg patient would require a 35-mg dose.

Newer commercially available NIR imaging systems, such as the SPY system (Novadaq Technologies Inc., Toronto, Canada), have been used recently in reconstructive surgery.^{30, 31} These early clinical experiences mirror the results seen in our study. There are major differences between the two imaging systems, however. The SPY system is laser-based while the FLARE™ system is LED-based; this may pose differences in eye safety in the operating room. More importantly, the FLARE™ imaging system acquires color video and NIR fluorescence images simultaneously and is capable of real-time overlay of the invisible NIR light images onto the color video images. This provides the surgeon with unambiguous surgical landmarks for image-guided surgery. The FLARE™ system also has the ability to quantify perforator perfusion metrics, as previously shown by our laboratory.^{14,15,32} The cost of parts in quantity 1 for the FLARE™ imaging system is ~\$120,000 USD. A miniaturized version of FLARE™, termed Mini-FLARE™, costs ~\$40,000 USD (Trojan et al., in review).

Although this is the first successful clinical pilot study with the FLARE™ system in reconstructive surgery, there are many areas that will need to be evaluated. Larger-scale studies need to be performed to assess clinical benefits. Each NIR angiography evaluation takes only two minutes, and a more effective decision-making process for perforator selection could potentially decrease overall operative times. Other benefits include the potential ability to prevent complications associated with poor perfusion. The costs associated with the additional use of this type of technology also need to be assessed. One limitation of the FLARE™ system is its 15-cm field of view: it is possible that flaps larger than this size would not be completely captured. For example, imaging was performed on each side of the abdomen, as the entire abdominal flap could not be captured with one scan. Finally, the surgeons in this study were blinded to the results of NIR imaging; in future studies we plan to have the surgeons directly apply the imaging results in perforator choice and flap design.

CONCLUSION

This is the first-in-human study demonstrating use of the FLARE™ system for NIR angiography in microsurgical perforator-flap breast reconstruction. It represents the successful translation of the technology from pre-clinical to clinical use. In this pilot study, an ICG dose of 4 mg per injection resulted in the highest observed CBR, SBR, and SNR relative to 2 mg and 6 mg doses; however, a larger study with sufficient statistical power is needed to confirm this result.

CLINICAL TRIAL REGISTRATION

The clinical study “Objective Flap Assessment During Reconstructive Surgery” is registered with the Clinical Trials registry at <http://www.clinicaltrials.gov>. The registration identification number is NCT00952107.

Acknowledgments

We thank Lorissa A. Moffitt and Mary McCarthy for editing, and Eugenia Trabucchi for administrative assistance. This work was funded by the National Institutes of Health grants R01-EB-005805 and R01-CA-115296 to JVF.

Sources of Funding: This study was funded by National Institutes of Health grants R01-EB-005805 and R01-CA-115296 to JVF.

REFERENCES

1. Giunta RE, Geisweid A, Feller AM. The value of preoperative Doppler sonography for planning free perforator flaps. *Plast Reconstr Surg* 2000;105:2381–2386. [PubMed: 10845290]
2. Masia J, Clavero JA, Larranaga JR, et al. Multidetector-row computed tomography in the planning of abdominal perforator flaps. *J Plast Reconstr Aesthet Surg* 2006;59:594–599. [PubMed: 16716952]
3. Rozen WM, Stella DL, Phillips TJ, et al. Magnetic resonance angiography in the preoperative planning of DIEA perforator flaps. *Plast Reconstr Surg* 2008;122:222e–223e.
4. Saint-Cyr M, Schaverien M, Arbique G, et al. Three- and four-dimensional computed tomographic angiography and venography for the investigation of the vascular anatomy and perfusion of perforator flaps. *Plast Reconstr Surg* 2008;121:772–780. [PubMed: 18317127]
5. Mathes DW, Neligan PC. Current techniques in preoperative imaging for abdomen-based perforator flap microsurgical breast reconstruction. *J Reconstr Microsurg* 2010;26:3–10. [PubMed: 20024888]
6. Chernyak V, Rozenblit AM, Greenspun DT, et al. Breast reconstruction with deep inferior epigastric artery perforator flap: 3.0-T gadolinium-enhanced MR imaging for preoperative localization of abdominal wall perforators. *Radiology* 2009;250:417–424. [PubMed: 19037016]
7. Uppal RS, Casaer B, Van Landuyt K, et al. The efficacy of preoperative mapping of perforators in reducing operative times and complications in perforator flap breast reconstruction. *J Plast Reconstr Aesthet Surg* 2009;62:859–864. [PubMed: 18490207]
8. Rosson GD, Williams CG, Fishman EK, et al. 3D CT angiography of abdominal wall vascular perforators to plan DIEAP flaps. *Microsurgery* 2007;27:641–646. [PubMed: 17941105]
9. Troyan SL, Kianzad V, Gibbs-Strauss SL, et al. The FLARE™ intraoperative near-infrared fluorescence imaging system: a first-in-human clinical trial in breast cancer sentinel lymph node mapping. *Ann Surg Oncol* 2009;16:2943–2952. [PubMed: 19582506]
10. Gioux S, Kianzad V, Ciocan R, et al. High-power, computer-controlled, light-emitting diode-based light sources for fluorescence imaging and image-guided surgery. *Mol Imaging* 2009;8:156–165. [PubMed: 19723473]
11. Lee BT, Matsui A, Hutteman M, et al. Intraoperative near-infrared fluorescence imaging in perforator flap reconstruction: current research and early clinical experience. *J Reconstr Microsurg* 2010;26:59–65. [PubMed: 20027541]

12. Matsui A, Lee BT, Winer JH, et al. Real-time intraoperative near-infrared fluorescence angiography for perforator identification and flap design. *Plast Reconstr Surg* 2009;23:125e–127e.
13. Matsui A, Lee BT, Winer JH, et al. Image-guided perforator flap design using invisible near-infrared light and validation with x-ray angiography. *Ann Plast Surg* 2009;63:327–330. [PubMed: 19692894]
14. Matsui A, Lee BT, Winer JH, et al. Submental perforator flap design with a near-infrared fluorescence imaging system: the relationship among number of perforators, flap perfusion, and venous drainage. *Plast Reconstr Surg* 2009;124:1098–1104. [PubMed: 19935293]
15. Matsui A, Lee BT, Winer JH, et al. Quantitative assessment of perfusion and vascular compromise in perforator flaps using a near-infrared fluorescence-guided imaging system. *Plast Reconstr Surg* 2009;124:451–460. [PubMed: 19644259]
16. Laird NM, Ware JH. Random-effects models for longitudinal data. *Biometrics* 1982;38:963–974. [PubMed: 7168798]
17. Littell, RC.; Milliken, GA.; Stroup, WW., et al. SAS for mixed models. Second Edition. SAS Institute, Inc.; Cary, NC: 2006.
18. Liang KY, Zeger SL. Longitudinal data analysis using generalized linear models. *Biometrika* 1986;73:13–22.
19. Winer JH, Choi HS, Gibbs-Strauss SL, et al. Intraoperative localization of insulinoma and normal pancreas using invisible near-infrared fluorescent light. *Ann Surg Oncol* 2010;17:1094–1100. [PubMed: 20033320]
20. Gibbs-Strauss SL, Nasr KA, Fish KM, et al. Nerve-highlighting fluorescent contrast agents for image-guided surgery. *Mol Imaging*. 2010 In Press.
21. Still J, Law E, Dawson J, et al. Evaluation of the circulation of reconstructive flaps using laser-induced fluorescence of indocyanine green. *Ann Plast Surg* 1999;42:266–274. [PubMed: 10096617]
22. Holm C, Mayr M, Hoftner E, et al. Intraoperative evaluation of skin-flap viability using laser-induced fluorescence of indocyanine green. *Br J Plast Surg* 2002;55:635–644. [PubMed: 12550116]
23. Holm C, Tegeler J, Mayr M, et al. Monitoring free flaps using laser-induced fluorescence of indocyanine green: a preliminary experience. *Microsurgery* 2002;22:278–287. [PubMed: 12404345]
24. Mothes H, Donicke T, Friedel R, et al. Indocyanine-green fluorescence video angiography used clinically to evaluate tissue perfusion in microsurgery. *J Trauma* 2004;57:1018–1024. [PubMed: 15580026]
25. Krishnan KG, Schackert G, Steinmeier R. The role of near-infrared angiography in the assessment of post-operative venous congestion in random pattern, pedicled island and free flaps. *Br J Plast Surg* 2005;58:330–338. [PubMed: 15780227]
26. Krishnan KG, Schackert G, Steinmeier R. Near-infrared angiography and prediction of postoperative complications in various types of integumentary flaps. *Plast Reconstr Surg* 2004;14:1361–1362. [PubMed: 15457083]
27. Yamaguchi S, De Lorenzi F, Petit JY, et al. The “perfusion map” of the unipedicled TRAM flap to reduce postoperative partial necrosis. *Ann Plast Surg* 2004;53:205–209. [PubMed: 15480004]
28. Holm C, Mayr M, Hoftner E, et al. Perfusion zones of the DIEP flap revisited: a clinical study. *Plast Reconstr Surg* 2006;117:37–43. [PubMed: 16404245]
29. Holm C, Mayr M, Hoftner E, et al. Interindividual variability of the SIEA Angiosome: effects on operative strategies in breast reconstruction. *Plast Reconstr Surg* 2008;122:1612–1620. [PubMed: 19050513]
30. Newman MI, Samson MC. The application of laser-assisted indocyanine green fluorescent dye angiography in microsurgical breast reconstruction. *J Reconstr Microsurg* 2009;5:21–26. [PubMed: 18925547]
31. Pestana IA, Coan B, Erdmann D, et al. Early experience with fluorescent angiography in free-tissue transfer reconstruction. *Plast Reconstr Surg* 2009;123:1239–1244. [PubMed: 19337092]
32. Matsui A, Lee BT, Winer JH, et al. Predictive capability of near-infrared fluorescence angiography in submental perforator flap survival. *Plast Reconstr Surg*. 2010 In Press.

Figure 1. The FLARE™ Imaging System

The cart-based imaging system is shown with major parts identified. A satellite monitor and footswitch are also available to the surgeon (not shown).

Figure 2. NIR Fluorescence Imaging after ICG Injection

A. Identification of perforating vessel (dashed circle) after injection of 2 mg (top), 4 mg (middle), or 6 mg (bottom) ICG on transferred skin flaps. Contrast was quantified using fluorescence intensity of the perforating vessel and background (solid circle).

B. Typical CBR curve after ICG injection. Signal peaks shortly after injection and gradually declines.

Figure 3. NIR Fluorescence Contrast per Dose ICG

A. Contrast-to-Background ratio (CBR), Signal-to-Background ratio (SBR), and Signal-to-Noise ratio (SNR) in each dose group. CBR was defined as $(I_{\text{perforator}} - I_{\text{background}}) / I_{\text{noise}}$, where I = fluorescence intensity. The 2-mg dose group differed significantly from the 4-mg and 6-mg dose groups on all measures (2 mg vs. 4 mg: CBR, $p = .001$; SBR, $p = .006$; SNR, $p = .001$. 2 mg vs. 6 mg: CBR, $p = .005$; SBR, $p = .025$; SNR, $p = .004$).

B. Effect of ICG dose on contrast after repeated injections.

C. Effect of ICG dose on background intensity after injection 2. After repeat injection of 6 mg ICG, the background intensity remains increased in comparison with 2-mg ($p = .009$) and 4-mg ($p = .008$) dose groups.

A.

B.

Figure 4. Selected perforator is not the NIR fluorescence dominant perforator

A. Quantification of the signal confirms the increase in fluorescence intensity of the selected perforator and decrease in intensity of the perforator that was not chosen.

B. Left and right sides of the abdomen are assessed before perforator dissection (two top rows) and the right side is chosen. Since the surgeon was blinded, NIR fluorescence information was not available for perforator selection. The perforator with the highest intensity (small arrow)

was not selected in favor of a less fluorescent perforator (large arrow). After perforator dissection (third row) and subsequent transfer (bottom row) the chosen perforator appears to gain intensity.

Table 1

Study Subject Characteristics

Subject	ICG dose (mg)	Age (yr)	Weight (kg)	Height (cm)	Body mass index	ADA Skin type	Pre-operative radiotherapy	Post-operative complications
1	2	29	84	173	28.1	II	No	Minor fat necrosis
2	2	47	66	163	24.8	III	No	None
3	4	64	77	168	27.3	II	No	None
4	4	56	70	160	27.3	II	No	Breast seroma
5	6	47	66	160	25.8	II	Yes	None
6	6	45	59	163	22.2	II	No	Minor skin loss
Mean		48.0 ± 11.8	70.3 ± 8.9	164.5 ± 5.1	25.9 ± 2.2			

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)

Table 2Contrast per Dose Group (Mean $\pm$ SD; Median)

Dose	Contrast-to-Background Ratio (CBR)	Signal-to-Background Ratio (SBR)	Signal-to-Noise Ratio (SNR)
2 mg	1.5 $\pm$ 0.3; 1.6	2.1 $\pm$ 0.2; 2.2	3.0 $\pm$ 0.3; 3.0
4 mg	4.2 $\pm$ 1.8; 3.9	3.6 $\pm$ 1.0; 3.5	6.0 $\pm$ 1.8; 5.7
6 mg	3.7 $\pm$ 1.7; 3.7	3.3 $\pm$ 1.2; 3.0	5.5 $\pm$ 1.7; 5.7

P-value from linear mixed-effects and GEE models: CBR (2 mg vs. 4 mg: 0.053, 2 mg vs. 6 mg: 0.0007, 4 mg vs. 6 mg: 0.604); SBR (2 mg vs. 4 mg: 0.084, 2 mg vs. 6 mg: 0.068, 4 mg vs. 6 mg: 0.539); SNR (2 mg vs. 4 mg: 0.028, 2 mg vs. 6 mg: 0.024, 4 mg vs. 6 mg: 0.628).