

Use of near-infrared fluorescence angiography with indocyanine green to evaluate direct cutaneous arteries used for canine axial pattern flaps

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Abstract

Objective: To describe the use of near-infrared angiography (NIRFA) to identify the vascularization of three canine axial pattern flaps (APFs) omocervical (OMO), thoracodorsal (THO), and caudal superficial epigastric (CSE); to establish a vascular fluorescence pattern (VFP) grading system; and to evaluate the effect of NIRFA on surgeon flap dimension planning compared to traditional landmark palpation (LP) and visualization assessments.

Study design: Experimental study.

Animals: A total of 15 healthy, client-owned dogs.

Methods: Dogs were sedated and flap sites were clipped. LP-based margins were drawn and preinjection images were recorded. Indocyanine green (ICG) was administered and VFP images were recorded. VFP scores were determined by five surgeons. Margin alterations were performed based on NIRFA-ICG images. Altered measurements were compared between LP and NIRFA-ICG images.

Results: Vascularization of the CSE flap was most visible with NIRFA with VFP scores 4/4 for 13/15 dogs. Intersurgeon agreement for VFP grades was poorest for THO (ICC = 0.35) and intermediate for OMO (ICC = 0.49) flaps. Surgeons were more likely to adjust dimensions for CSE flaps relative to OMO (OR 17.3, 95% CI: 6.2, 47.8) or THO (25.5; 8.6, 75.7).

Conclusion: Using a grading system, we demonstrated that the CSE flap was most visible. Surgeons were more likely to adjust the LP-CSE flap margins

Abbreviations: APFs, Axial pattern flaps; CI, confidence interval; CSE, caudal superficial epigastric; ICC, interclass correlation coefficients; ICG, indocyanine green; IQR, interquartile range; LP, landmark palpation; NIRFA, Near-infrared angiography; OMO, Omocervical; OR, odds ratio; SD, standard deviation; THO, Thoracodorsal; T_i, time to fluorescence; T₀, time of injection; T_{max}, time to maximum fluorescence; VFP, vascular fluorescence pattern.

based on fluorescence patterns and were more likely to rely on LP when visualization scores were low.

Clinical significance: NIRFA has possible applications identifying some direct cutaneous arteries of APFs and their associated angiosomes in real-time. Further investigation is indicated to study NIRFA's potential to improve patient specific APF planning.

1 | INTRODUCTION

Axial pattern flaps (APFs) are typically chosen for their sturdy vascular supply compared to other local flaps.^{1–3} Surgical planning of APFs relies on palpating anatomic landmarks to ensure proper incorporation of the vascular angiosome. However, using landmarks established in a limited number of dogs for both dogs and cats may lead to inadequate blood supply, resulting in the reported high rates of partial flap necrosis of 29%–70% in recent canine and feline studies.^{4–7} Variability in vessel size and location can hinder optimal flap development when landmark palpation (LP) and visualization alone are used.

Doppler flowmetry,^{8–10} intraoperative fluorescein injections,^{11,12} and imaging angiography¹³ have been described to improve preoperative planning and intraoperative vessel identification. However, each technique carries its own limitations including sensitivity to small movements, need for complete, intraoperative darkness, and difficulty performing them in real-time. The ideal intraoperative imaging technique would be minimally invasive, rapid, nontoxic, and easy to interpret.

Near-infrared fluorescence angiography (NIRFA) with intravenous indocyanine green (ICG) uses near-infrared light to observe ICG in real-time within the vasculature. While NIRFA has been effective in humans for preoperative planning and intraoperative evaluations of skin flap vascular integrity,^{14–19} its use in veterinary medicine has been limited.^{20–23} Only two case reports, one involving a feline and one a canine, have described the use of NIRFA-ICG for visualizing perforating vessels in APFs.^{24,25} The use of NIRFA-ICG for APF vessel visualization prior to and during flap elevation resulted in improved surgeon awareness of intraoperative vessel location.^{24,25} These observations suggest that NIRFA-ICG has the potential to improve APF planning and flap survival in canine patients.

The objectives of this study were to (1) describe NIRFA-ICG for real-time identification of direct cutaneous arteries of three APFs and their associated angiosomes in dogs, (2) describe vascular fluorescence patterns (VFP) using a simple grading system, and (3) evaluate effect on surgeon decision-making with respect to alteration of APF margins after traditional

LP-based assessments. We hypothesized that NIRFA-ICG would (1) allow for noninvasive visualization of all evaluated APF angiosomes and (2) would influence surgeons to change APF dimensions compared to LP-based margins. This investigation was a randomized proof of concept evaluation to determine if VFP grading is feasible and clinically practical. Criteria for clinical feasibility were visualization of the targeted angiosome via NIRFA and ability to grade VFP; secondary evaluation was if visualization affected surgeon behavior, evaluated by change in flap margins relative to LP-based assessment.

2 | MATERIALS AND METHODS

2.1 | Animals

The study was approved by the Institutional Animal Care and Use Committee (no. 202011209) and college internal review board (no. 2020–23). From April 2021 to June 2022, veterinary student-owned dogs were enrolled after obtaining owner consent and a full history. No participation incentives were offered to the owners. Inclusion criteria consisted of a normal physical examination and chemistry panel. Exclusion criteria were presence of active skin lesions, known skin allergies, and abnormal renal and/or hepatic values on presedation bloodwork.

2.2 | Imaging procedures

Dogs were catheterized and sedated with intravenous (IV) acepromazine (0.005–0.02 mg/kg) and butorphanol (0.1–0.2 mg/kg) and positioned in right lateral recumbency. Hair was clipped using a no. 10 clipper blade (Andis, Sturtevant, Wisconsin) following standard preoperative recommendations. Anatomic landmarks for each axial pattern flap (APF), caudal superficial epigastric (CSE) omocervical (OMO), and thoracodorsal (THO), were visualized and palpated by a single evaluator (SNE) to determine the APF margins.^{3,26} LP-based margins were drawn using a surgical skin marker (Securline, Caledonia, Michigan) and preinjection images were

obtained for each flap. Indocyanine green (ICG, 25 mg vials, Stryker California) was diluted to a 2.5 mg/mL concentration and administered IV at 0.5 mg/kg. Immediately following ICG administration, images were captured with a SPY-Portable Handheld Imager (SPY-PHI Elite, Stryker, Kalamazoo, Michigan). The SPY-PHI camera utilizes a laser to excite the ICG at 20–40 pulses/s with an energy output (maximum) of 2 millijoules per pulse. The system has an emission filter wavelength of 805 nm. The camera was stabilized by a custom-made rig distanced 30–60 cm from the skin surface of each dog. All images were calibrated with an embedded 10 mm diameter marker (Arrowhead Forensics, Lenexa, Kansas) and obtained in the presence of ambient light. Time of injection (T_0), time to fluorescence (T_i), and time to maximum fluorescence (T_{max}) were determined using the SPY-PHI. OMO and THO flaps were imaged simultaneously due to their location. Dogs were repositioned in dorsal recumbency within 2 min of the initial injection to visualize the CSE flap. During and after image collection, the following parameters were monitored: pulse oximetry, heart rate, respiratory rate, temperature, and blood pressure.

2.3 | Video image assessment and vascular fluorescence pattern scoring

Five diplomates of the American College of Veterinary Surgeons (JB, PR, BC, KH, MM) evaluated images independently for each dog. The images were presented to the surgeons in a randomized order (<https://www.randomizer.org/>) with the same APF order (OMO, CSE, THO). The five surgeons evaluated each flap on 15 dogs, resulting in 75 evaluations per flap. After watching an instructional video, participants were provided with both video clips and single still images of each flap for initial injection (OMO and THO flap) or post-injection (CSE flap) images. Single still images were selected based on highest level of fluorescence. Images were viewed on an online platform (YouTube, San Bruno, California). Each participant independently graded the visibility of the vascular fluorescence pattern VFP for each flap on each dog on a five-point scale (Figure 1): 0 – no identifiable VFP, 1 – indistinct VFP inconsistent with the target vessel, 2 – indistinct VFP consistent with the target vessel, 3 – distinct VFP consistent with the primary target vessel, 4 – distinct VFP consistent with the primary target vessel to the level of tertiary branches. Surgeons were able to repeatedly review the videos at their discretion prior to deciding. Observers were then asked if they would adjust LP-based APF margins based on the corresponding NIRFA image. Alterations were made manually with a marker on overlaid physical copies of the image (Figure 2).

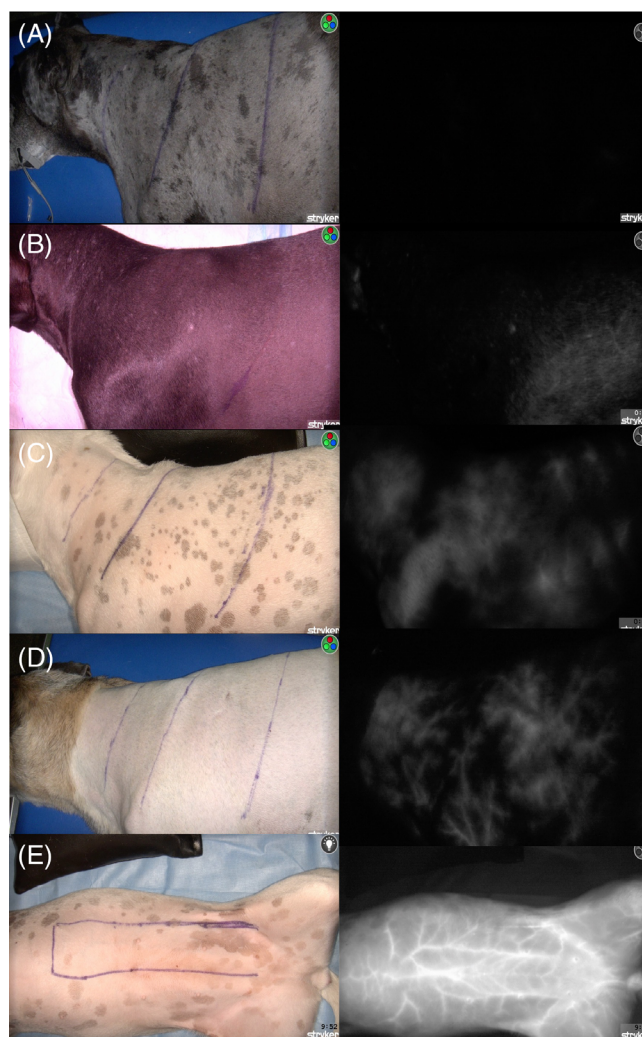


FIGURE 1 Examples of different vascular fluorescence pattern (VFP) grades. (A) 0 – no identifiable VFP. (B) 1 – indistinct VFP inconsistent with the target vessel. (C) 2 – indistinct VFP consistent with the target vessel. (D) 3 – distinct VFP consistent with the primary target vessel. (E) 4 – distinct VFP consistent with the primary target vessel to the level of tertiary branches.

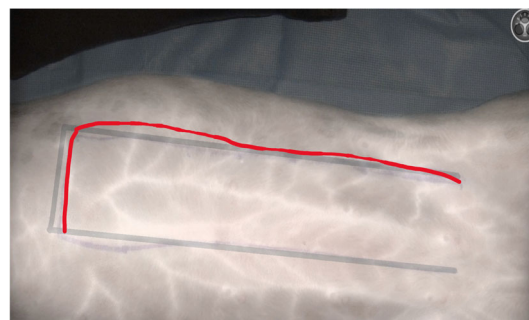


FIGURE 2 Alterations between landmark palpation (LP) based and near-infrared fluorescence angiography with ICG (NIRFA-ICG) images for the caudal superficial epigastric (CSE) flap. The gray line represents the LP-based margins for the CSE flap while the red line depicts a surgeon observer's alterations based on the NIRFA-ICG image.

2.4 | Image analysis

Images were imported into a computerized planimetric analysis program (ImageJ, U.S. National Institutes of Health, Maryland). Flap dimensions (area, length, width) were computed to the nearest 0.1 cm for both LP-based and NIRFA images.

2.5 | Statistical analysis

2.5.1 | Study design

This was a descriptive, proof of concept, inter-rater reliability study.

2.5.2 | Sample size

With five surgeon evaluators, approximately 18 dogs were required to estimate an inter-rater correlation of 0.85 with a confidence interval (CI) width of 0.2.²⁷

2.5.3 | Analyses

The primary outcomes were VFP score (0–4) and dimension alteration (yes/no; defined as any alteration of flap area, linear dimensions, and/or direction of approach). Secondary outcome variables were flap area (cm²), flap length (cm), and flap width (cm). A clinically relevant change in flap dimensions from LP standard was determined to be

±10% difference in linear dimension or 20% change in flap area from the corresponding LP reference.

All data were analyzed in SAS version 9.4 (SAS Inc, Cary, North Carolina). Summary statistics (mean and standard deviation, SD) were calculated for continuous variables (dog age and weight, fluorescence times, flap dimensions) in SAS proc means. Scores were summarized by counts for each score category.

Differences between surgeons for VFP scores were evaluated for each flap type by ordinal logistic regression (SAS proc glimmix).²⁸ Reliability for VFP ratings of all dogs measured by the same fixed set of raters was assessed by the Shrout-Fleiss intraclass correlation coefficients (ICC) for fixed number of raters, *k*. The ICC is the ratio of the (variance of interest) / (total variance). In summary, ICC >0.75 indicated good interrater reliability (low within-rater variation and large between-rater variation), ICC between 0.4 and 0.75 moderate reliability, and ICC <0.4 poor reliability (large within-rater variation and low between-rater variation). However, if both within- and between-rater variation were very small, the ICC approached zero and could not be used as a single measure of reliability; interpretation was then based on the VFP scores alone.²⁹

ICCs were calculated for each flap type by two-way random-effects analysis of variance (SAS proc glm)^{30,31} with corresponding exact 95% confidence intervals.²⁷ The probability of flap size alteration following visualization by NIRFA (“change” yes/no) was quantified by odds ratios, using generalized linear models for binary outcomes (SAS proc glimmix).²⁸ Changes in flap dimensions (length, width, area) were estimated for each flap type with mixed-model analysis of variance (SAS proc mixed)

TABLE 1 Signalment information for the 15 enrolled dogs.

Dog	Gender and reproductive status	Breed	Bodyweight (kg)	Hair color	Age (years)
A	Female Spayed	Mixed breed	9	White, fawn	5.4
B	Female Spayed	Mixed breed	22.7	Black	1.7
C	Female Spayed	Mixed breed	14.9	Gray	3.5
D	Male Castrated	Mixed breed	32	Black, white	6.2
E	Male Castrated	Mixed breed	14.7	Gray	1.8
F	Female Spayed	Mixed breed	21.7	Gray	3.9
G	Female Spayed	Mixed breed	19.2	Brindle	5.7
H	Female Spayed	Mixed breed	9.2	Cream	2.8
I	Male Castrated	Mixed breed	42.7	Gray	11.6
J	Female Spayed	Labrador Retriever	24.4	Black	2.9
K	Female Spayed	Bull Terrier	25.3	Black/white	8.2
L	Female Spayed	Longhaired Dachshund	5.3	Black/tan	11.6
M	Male Castrated	Mixed breed	33	Sable	9.8
N	Male Castrated	Mixed breed	20.2	Black/tan	3.0
O	Female Spayed	Mixed breed	19.1	Gray	6.7

with surgeon as fixed factor and the pertinent LP dimension as the reference value.²⁸

3 | RESULTS

3.1 | Animals

A total of 15 dogs met the inclusion criteria and were enrolled. Dog reproductive statuses included eight spayed

female and seven castrated males. Breeds included mixed breed (12), Labrador Retriever (1), Bull Terrier (1), and Dachshund (1) (Table 1). Median age was 5 years (IQR 2.7; range 1–11) and bodyweight was 20 kg (IQR 15, 25; 5–43).

3.2 | Fluorescence times

Average T_i was 11 s (SD 2.3) and average T_{max} was 16 s (SD 4).

3.3 | VFP scores

The CSE flap was the most readily visualized with VFP scores of 4/4 for 13/15 dogs (Figure 3). Excellent NIRF visualization was obtained for all proposed CSE flaps (73/75 observations with VFP score 4) (Table 2). For OMO and THO flaps, 45/75 (60%) and 36/75 (48%), respectively, of VFP observations were less than 2. The lowest overall VFP scores were for OMO flaps, with 19% having no discernible VFP (Table 2).

3.4 | Surgeon VFP agreement

For the CSE flaps, VFP visualization was high (73/75, 97% VFP with score 4) for all five surgeons across all 15 dogs. As a result, CSE flap ICC was close to zero (0.09). Visualization was less consistent for THO and OMO flaps. Rater reliability for THO flaps was ICC = 0.35 (95% CI: 0.12, 0.59) and 0.49 (95% CI: 0.24, 0.70) for OMO flaps.

3.5 | Margin adjustments

Average LP-based flap lengths, widths, and areas are shown in Table 3 and alterations to flap dimensions after NIRFA viewing are demonstrated in Table 4. Surgeons adjusted flap margins for CSE most often after viewing NIRFA images, altering margins of 49 (65%) of 75 flap images after visualization (Figure 4). For the OMO flap images, only 2/45 (4%) were altered if VFP scores were <2, compared to

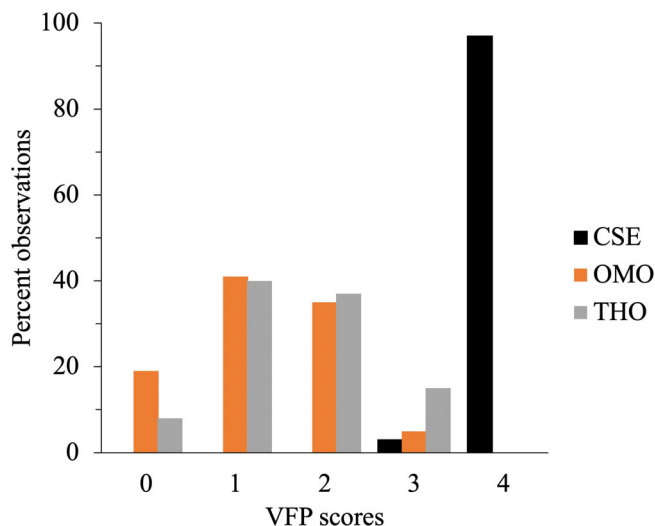


FIGURE 3 Distribution of vascular fluorescence pattern (VFP) scores for each axial pattern flap: caudal superficial epigastric (CSE) in black, omocervical (OMO) in orange, and thoracodorsal (THO) in gray.

TABLE 2 Vascular fluorescence pattern scores for five surgeons evaluating 15 dogs for each of three flaps.

VFP score	CSE	%	OMO	%	THO	%
0	0	0	14	19	6	8
1	0	0	31	41	30	40
2	0	0	26	35	28	37
3	2	3	4	5	11	15
4	73	97	0	0	0	0

Note: Percentages are based on the number of scores in each category for a total 75 observations for each flap.

Abbreviations: CSE, caudal superficial epigastric; OMO, omocervical; THO, thoracodorsal; VFP, vascular fluorescence pattern.

TABLE 3 Flap linear dimensions (length, width; cm) and area (cm²) obtained for the reference LP method (reference standard; mean, SD) for three flap sites.

LP-based dimensions	CSE	OMO	THO
Flap length, cm (mean, SD)	31.3 (4.6)	11.7 (2.5)	12.7 (2.6)
Flap width, cm (mean, SD)	5.7 (1.4)	5.1 (1.3)	9.0 (1.7)
Flap area, cm ² (mean, SD)	170.8 (47.1)	66.9 (34.3)	120.1 (45.3)

Note: Dimensions are averaged for 15 dogs.

Abbreviations: CSE, caudal superficial epigastric; LP, landmark palpation; OMO, omocervical; SD, standard deviation; THO, thoracodorsal.

TABLE 4 Surgeon-specific APF changes following NIRFA visualization for three flap sites.

Flap	CSE			OMO			THO		
Surgeon	Relative mean change from LP (cm), 95% CI			Relative mean change from LP (cm), 95% CI			Relative mean change from LP (cm), 95% CI		
Flap length, cm									
A	0	−1.7	1.7	−1.1	−1.8	−0.4	−0.5	−1.4	0.5
B	0	−1.9	1.9	0	0	0	0	0	0
C	−6	−7.7	−4.2	−1.2	−2.1	−0.3	−0.7	−3.4	2.1
D	−3.5	−6.2	−0.8	0	0	0	0	0	0
E	−5.2	−7.1	−3.2	−1.4	3.4	0.6	−1	−3.1	1
Surgeon	Relative mean change from LP (cm), 95% CI			Relative mean change from LP (cm), 95% CI			Relative mean change from LP (cm), 95% CI		
Flap width, cm									
A	1.8	1	2.6	0.9	0.2	1.6	0.2	−1.1	1.6
B	1	0.1	1.9	0	0	0	0	0	0
C	−0.3	−1.1	0.5	0.3	−0.6	1.3	−0.7	−4.3	3.1
D	−0.1	−1.4	1.2	0	0	0	0	0	0
E	−1	−1.9	−0.1	−0.7	−2.6	1.2	−0.7	−3.5	2
Surgeon	Relative mean change from LP (cm ²), 95% CI			Relative mean change from LP (cm ²), 95% CI			Relative mean change from LP (cm ²), 95% CI		
Flap area, cm ²									
A	34	20.6	47.5	−8.9	−20.7	2.9	−22.4	−38.5	−6.3
B	2.1	−13	17.2	0	0	0	0	0	0
C	−45.4	−59.6	−31.2	−16.1	−32.2	0.1	−33.5	−80.2	13.1
D	−23.4	−45.1	−1.7	0	0	0	0	0	0
E	−45.2	−61	−29.5	−15.5	−49.3	18.3	−23.4	−58.2	11.3

Note: Changes are depicted as relative mean change from the LP-based reference value shown in Table 3 (95% CIs), averaged over 15 dogs.

Abbreviations: APF, axial pattern flap; CI, confidence interval; CSE, caudal superficial epigastric; LP, landmark palpation; NIRFA, near-infrared angiography; OMO, omocervical; THO, thoracodorsal.

flaps with VFP scores ≥ 2 , with 15/30 (50%) being altered. For the THO flap images, none were altered when VFP scores were < 2 , but 14/39 (36%) were changed for VFP scores ≥ 2 . Odds ratios (OR) for alterations indicated surgeons were more likely to adjust CSE flaps relative to both THO (OR 25.5 [95% CI: 9.3, 86.6]) and OMO (OR 17.3 [95% CI: 6.6, 53.2]) flaps. The odds of surgeon alteration of OMO flaps relative to THO flaps was 1.5 (95% CI: 0.5, 4.1).

For CSE flap length, surgeons C, D, and E altered the flap by $> 10\%$ of the LP-based mean length (> 3.13 cm) electing to shorten the flap. For OMO flap length, surgeons C and E changed the flap length by $> 10\%$ of the LP-based mean length (> 1.17 cm) electing to shorten the flap. Surgeons did not adjust THO flap length by a clinically relevant amount.

For CSE flap width, surgeons A, B, and E altered the flap $> 10\%$ of the LP-based mean width (> 0.57 cm). Two surgeons widened while one narrowed the CSE flap. For OMO flap width, surgeons A and E changed the flap by

$> 10\%$ of the LP-based mean width (> 0.51 cm). One surgeon widened the OMO flap while the other narrowed it. Surgeons did not adjust THO flap width by a clinically relevant amount.

For CSE flap area, surgeons C and D altered the area by at least 20% of the LP-based mean area (34.16 cm²), electing to decrease it. For OMO flap area, surgeons C and D changed the flap by at least 20% of the LP-based mean area (13.38 cm²), electing to decrease it. For THO flap area, surgeon C changed the flap by at least 20% of the LP-based mean area (24.02 cm²), electing to decrease it.

4 | DISCUSSION

We used NIRFA and a grading scheme to identify direct, cutaneous angiosomes in three commonly used APFs in a diverse group of dogs. In this canine sample, the CSE flap had the highest VFP grading compared to OMO and THO

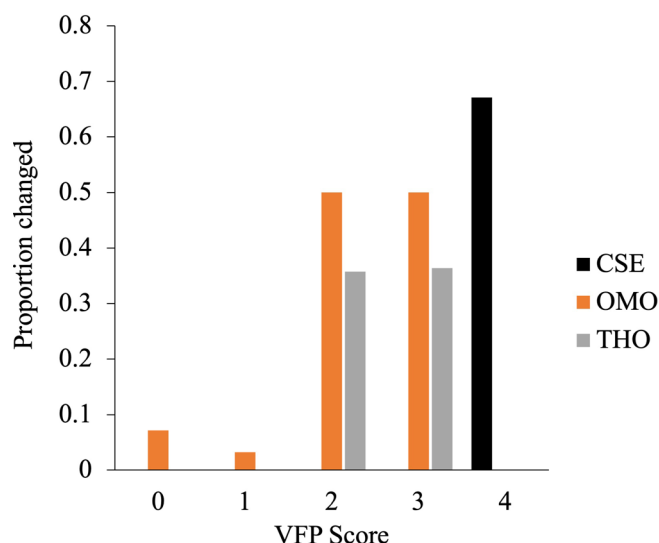


FIGURE 4 Proportion of margins for each flap adjusted from the margins established by landmark palpation after near-infrared fluorescence visualization. Five surgeons evaluated three flaps on 15 dogs for a total of 75 observations per flap: caudal superficial epigastric (CSE) in black, omocervical (OMO) in orange, and thoracodorsal (THO) in gray.

flaps and a higher surgeon agreement. When shown the same 15 NIRFA-CSE flap images, surgeons agreed overall on the VFP scores. Agreement was less consistent for the OMO and THO flaps, which is likely attributed to the difficulty in visualizing the angiosomes and therefore low VFP scores. Based on our results, we partially accept the first hypothesis as only the CSE flaps were consistently visualized with VFP scores greater than 3. Although NIR light can penetrate as deep as 3 cm,¹⁹ NIR imaging reportedly has difficulty capturing structures greater than 5–8 mm deep.³² Holt et al. observed challenges in preoperative NIR imaging of tumors with intact skin.³³ Higher VFP grades and agreement associated with the CSE flaps may be due to the lack of any panniculus muscular layer over the perforating vessel compared to the OMO and THO flaps (e.g., presence of the cutaneous trunci) or that the OMO and THO vessels and respective angiosomes are deeper than the CSE vessels.

We partially accept our second hypothesis as surgeons were more likely to adjust CSE flap margins after viewing NIRFA images compared to OMO and THO flaps. Surgeons in this study were more likely to alter APFs when they could visualize the angiosome more clearly (VFP score ≥ 2) and were more likely to rely on LP-based APF margins when they could not (VFP scores < 2). Although surgeons overall were more likely to adjust CSE flap margins, they were not consistent in the direction of changes made (Table 4). Often, surgeons were more likely to shorten the CSE and OMO flap lengths and decrease the overall flap area of all three flaps; however, no other

trends were consistent. Clinical applications of NIRFA-ICG are necessary to determine if VFP visualization would influence surgeons' margins.

The Dachshund (Dog L) and Labrador Retriever (Dog J) had the lowest overall VFP scores, suggesting that they were the most difficult to image. We suspect that these low scores may be associated with interference related to optical properties of the dogs' skin and fur (both had black fur). Future studies with a larger population of dogs and a greater diversity of fur colors are needed to test this hypothesis.

We piloted a VFP grading system when using NIRFA to evaluate direct, cutaneous angiosomes for three commonly used APFs. While we report the first grading scheme to describe NIRFA with APFs in veterinary medicine, other grading schemes have been described using ICG for various other target tissues such as visualization of the thoracic duct (0–3).²² Our goal with establishing the VFP grading system was to better standardize the visualization of the associated angiosomes. Real-time quantification of fluorescence intensity can be obtained using ICG-NIRF such as with the SPY-Q analysis post-processing software (SPY-PHI, Stryker, Kalamazoo, Michigan); however, a positive, control organ such as the liver is necessary. Due to the difficulty of establishing a quantification control on skin, the VFP grading system may be more practical when evaluating APFs using NIRFA. Additional studies comparing the current gold standard control of liver control to normal skin are necessary.

When planning APF margins, it is vital to maintain the perforating angiosome to ensure flap survival. Recent literature reports rates of necrosis to be 29% with canine CSE flaps⁴ and up to 70% for canine THO flaps.⁷ These rates of necrosis may be secondary to inadequate vasculature visualization and failure to preserve the perforating angiosome of interest. Matsui et al. demonstrated that immediate use of NIRFA after submental perforator flap creation in pigs accurately predicted areas of perfusion at 72 h compared to the initial clinical assessment of the flaps.¹⁴ They described NIRFA findings of insufficient arterial perfusion after immediate flap creation corresponded with histologic evidence of necrosis, further supporting the use of NIRFA-ICG intraoperatively.¹⁴ Holm et al. similarly described the use of NIRFA as a sensitive method for assessing blood flow of pedicle skin flaps with intraoperative filling defects correlating with decreased areas of perfusion.¹⁷ The use of NIRFA may allow for patient-specific APF planning intraoperatively and may improve flap survival compared to flaps relying solely on LP-based margins.

When administered IV, ICG initially remains confined to the vascular compartment through its binding

with plasma proteins, making it an ideal imaging agent for perfusion studies and angiography. Visualization of ICG occurred rapidly in this study, with peak fluorescence occurring on average 11 s after injection. In previous animal studies, ICG was identified within 20 s (porcine model)³⁴ and 15 s (feline)²⁴ of initial injections. We showed the possibility of real-time, direct visualization of the APF angiosomes without significantly increasing patient time under sedation.

There is no recommended IV dose for NIRFA ICG to date in dogs, likely due to the scarcity of veterinary clinical reports. A 2.5 mg dose (0.54 mg/kg and 0.29 mg/kg ICG IV) was used in a case report evaluating NIRFA for caudal auricular flaps in two cats.²⁴ A dose of 0.5 mg/kg ICG IV was used in another study evaluating NIRFA for the superficial brachial flaps in two dogs.²⁵ In human medicine, the recommended maximum dose of ICG is 2 mg/kg per the manufacturer. Doses in humans can vary significantly, however most papers report ICG-angiography dosing regimens for reconstructive flap surgeries to range from 2.5–25 mg for fixed doses and 0.025–0.5 mg/kg for weight adjusted doses.³⁵ Our selection of 0.5 mg/kg IV ICG was based on these reports. In our clinical experience, doses higher than 0.5 mg/kg ICG IV resulted in a quenching effect, or a decrease in fluorescence intensity,³⁶ during wound assessments, obscuring vessel visualization. Based on the consistently high VFP scores with CSE APFs, we recommend using this dose for angiosome visualization. Future studies optimizing the dosing and timing of ICG for use with NIRFA in veterinary patients would be incredibly valuable.

The main limitation of this study was the use of two-dimensional imagery to represent a three-dimensional patient. The total measurements (area, length, and width) obtained from the LP-based and NIRFA images may have underestimated the measured values. Another limitation was the generation of the LP-based margins. These were not determined by the reviewing surgeons and were not generated by a board-certified surgeon. However, a boarded certified surgeon supervised the LP-based landmarks. These procedures were necessitated by safety restrictions by COVID19 during the time of data acquisition. Therefore, surgeons were asked to make critical adjustments of the skin margin assessment without having palpated the patient for themselves. Simple movements, such as slight abduction of the limb, can drastically alter the positioning and the skin “margins” for APFs. Because surgeons were not physically present for the palpations or the real-time viewing of the NIRFA-ICG, their interpretation of the digital copies of the VFPs may not truly represent their adjustments had they been in person for the image collection.

Additionally, surgeons were presented with digital copies of the VFP. Post-viewing comments included the need to replay video clips and revisit images on the provided website prior to selecting their final VFP scores. We did not test for with-in surgeon repeatability of VFP evaluations, which may have affected surgeon interpretation and evaluation of the LP-based and NIRFA images.

The presence of ambient light may have impacted the collected data in this study. Chiti et al. has demonstrated that higher values of mean signal-to-background ratios and lower limit of detections of fluorescence are obtained in dark conditions for the two fluorescence camera systems tested.³⁷ Although no overhead surgical lights were on nor pointed at the imaged fields, the room lights were activated during image collection. It is possible that the presence of ambient light decreased the true visible fluorescence and therefore could impact the reported VFPs. All dogs were imaged in the same artificial light conditions.

Although the dogs' temperature, blood pressure, and heart rate were monitored during their sedation, these values were noted but not reported in this study. The exclusion of these values, which can impact vascular perfusion and therefore vessel identification during angiography, is another limitation of this study. Other limitations were the small number of observers that were available to assess images and the small size of the study conducted at a single center, thus limiting the study's generalizability. Although we pre-emptively determined that 18 dogs would be needed for significance assessment, only 15 were enrolled due to participant availability.

VFP scores may have been affected by image capture distance. Manufacturer recommendations suggest positioning the imager 10–40 cm from the surface or tissue. Images were obtained at different distances during data collection depending on the size of the dog. However, visualization of VFPs became more difficult the further the device was from the target tissue. Skin preparation process may have also affected VFP scores. A #10 blade was used to clip hair in all patients because #40 clippers were noted to create unpredictable frequencies of superficial skin trauma or razor burn (unpublished, preliminary data). However, longer fur length than is typical for #40 clippers may have influenced the captured VFP images by obscuring the angiosomes, and thus falsely lowering the possible VFP scores.

With this preliminary data, we suggest that NIRFA can be used for real-time identification of direct cutaneous arteries of some APFs and their associated angiosomes, with the potential to improve patient specific APF planning in canine surgeries. Future studies involving the intraoperative use of NIRFA for APF planning are necessary to evaluate the true clinical utility (e.g.,

improved overall flap survival, lower complications, etc.) of using this technology.

AUTHOR CONTRIBUTIONS

Eiger SN, VMD: Substantially contributed to study concept and design, data collection, data analysis, data interpretation, writing, drafting, revising, and approving the final manuscript. Bertran J, DVM, MS, DACVS (Small Animal): Substantially contributed to data collection (surgeon observer), data analysis, data interpretation, writing, drafting, revising, and approving the final manuscript. Reynolds P, PhD: Substantially contributed to data analysis for statistical significance, data interpretation, writing, drafting, revising, and approving the final manuscript. Regier P, MS, DACVS (Small Animal): Substantially contributed to data collection (surgeon observer), drafting, revising, and approving the final manuscript. Case JB, DVM, MS DACVS (Small Animal): Substantially contributed to data collection (surgeon observer), drafting, revising, and approving the final manuscript. Ham K, DVM, MS, DACVS (Small Animal): Substantially contributed to data collection (surgeon observer), drafting, revising, and approving the final manuscript. Mison M, DVM, DACVS: Substantially contributed to data collection (surgeon observer), drafting, revising, and approving the final manuscript. Fox-Alvarez WA, DVM, MS, DACVS (Small Animal): Substantially contributed to study concept and design, data collection, drafting, revising, and approving the final manuscript.

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CONFLICT OF INTEREST STATEMENT

This manuscript represents a portion of a thesis submitted by Sophie N. Eiger to the University of Florida Graduate Program as a partial fulfillment of the requirements for a Master of Science degree. The authors declare no conflict of interest related to this report. Results of this study were presented at the ACVS 2023 Summit on October 12, 2023.

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