Exploring the clinical applicability of quantitative near-infrared fluorescence imaging with indocyanine green: towards objective and standardized assessment of tissue perfusion

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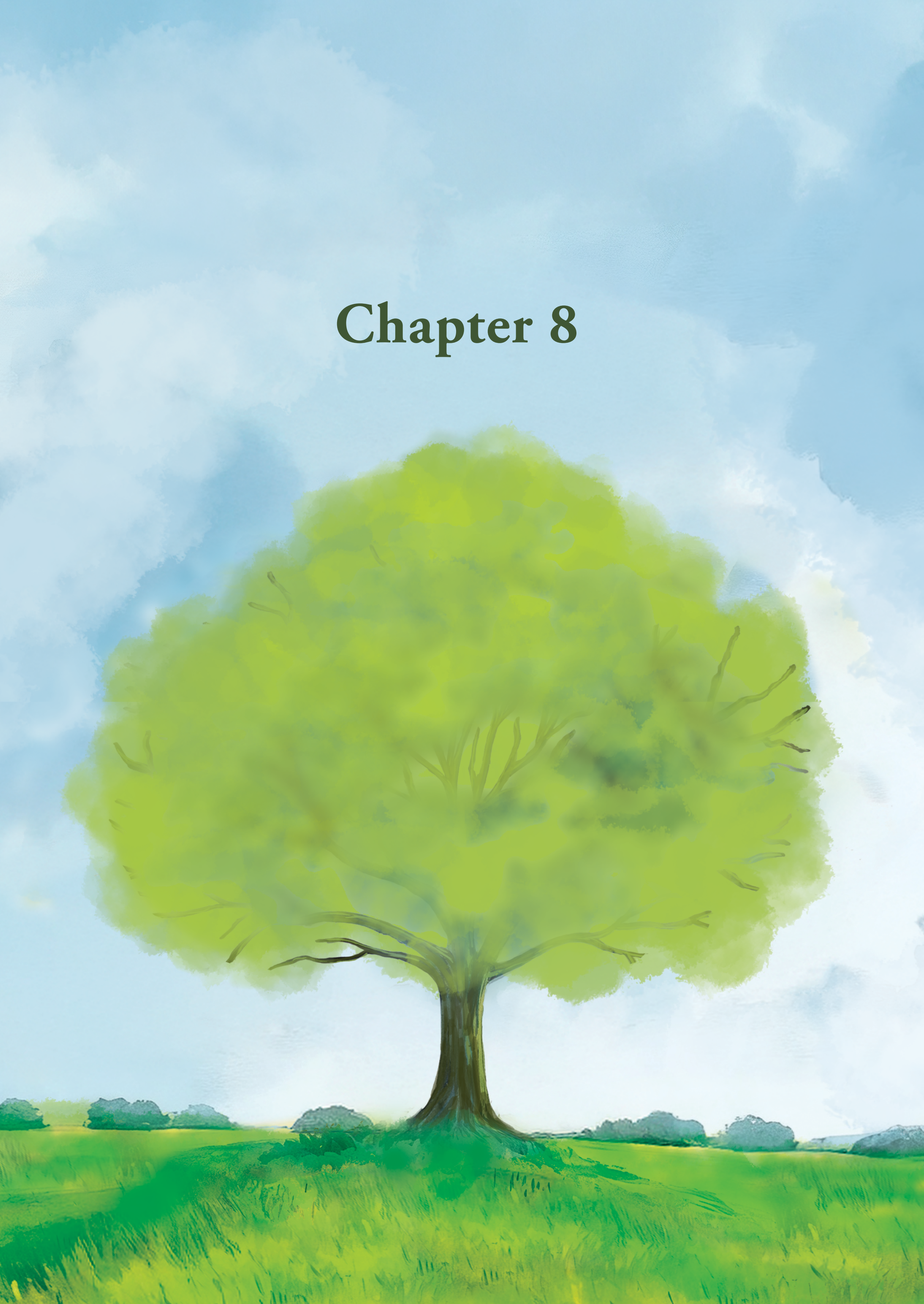
Chapter 8



Summary

Discussion and future perspectives

Summary

Dreaded complications in surgery, such as anastomotic leakage, flap necrosis, and non-healing ulcers, remain an important source of morbidity and are all strongly linked to inadequate tissue perfusion. Conventional diagnostic tools provide information on macrovascular level, but do not capture local perfusion, which is most directly related to tissue viability. Near-infrared fluorescence (NIRF) imaging with indocyanine green (ICG) enables real-time visualization of local, microvascular blood flow, and has been increasingly applied across surgical disciplines. However, interpretation of the fluorescence signal remains predominantly visual, making it qualitative and subject to interobserver variability. Quantitative analysis of the fluorescence signal has therefore been proposed to provide objective and reproducible assessment of tissue perfusion. The aim of this thesis was to evaluate the feasibility, clinical applicability, and standardization of quantitative NIRF imaging with ICG across multiple surgical domains in order to bring this technique closer to clinical implementation, as outlined in **Chapter 1.**

First off with vascular surgery, **Chapter 2** evaluates the potential of quantitative NIRF imaging to predict clinical outcome after revascularization procedures in patients with lower extremity arterial disease (LEAD). Perfusion images were performed before and after intervention, and the changes in quantified parameters were correlated to clinical improvement. Patients in whom clinical improvement was observed showed significant differences in perfusion parameters, whereas those without clinical improvement did not. This finding indicates that quantitative NIRF imaging may provide predictive value for revascularization success and enable timely adaptation of treatment when required. These findings relate to the broader question of how revascularization procedures can be optimized, which was further explored in **Chapter 3**. In this study, the angiosome concept was examined, which assumes that direct revascularization of the wound-related angiosome would provide superior clinical outcomes. Quantitative perfusion changes were assessed in directly and indirectly revascularized angiosomes. Significant changes of perfusion parameters were observed in both groups, with consistent results across subgroups of patients with chronic limb-threatening ischemia and within endovascular and surgical techniques. In addition, significant collateral were very frequently observed in indirectly revascularized angiosomes. These findings suggest that the angiosome concept may not be applicable in patients with LEAD and therefore revascularization strategy should not solely focus on creating in-line flow to the affected angiosome.

Chapters 4 and **5** share the aim of evaluating quantitative perfusion assessment in gastrointestinal surgery, focusing on tissue at risk of anastomotic leakage. In **Chapter 4**, intraoperative imaging was performed on bowel segments after transection of the vascular supply, but before resection.

In **Chapter 5**, intraoperative imaging was performed after creation of the gastric conduit, immediately before construction of the cervical anastomosis. In both studies, three comparable perfusion patterns were identified, potentially corresponding to adequate perfusion, a transition zone, and poor perfusion, although larger studies are needed to establish their relationship with anastomotic leakage risk. Interobserver agreement for subjective assessment of fluorescence signal in both studies was poor to moderate. These findings demonstrate that quantitative NIRF imaging is feasible in both colorectal and oesophageal surgery, and emphasize that subjective interpretation is highly variable, underlining the potential of quantification to support the prevention of anastomotic leakage.

In **Chapter 6**, the study protocol of a multicenter randomized controlled trial is presented, evaluating whether the use of NIRF imaging can reduce fat necrosis after deep inferior epigastric perforator (DIEP) and muscle-sparing transverse rectus abdominis myocutaneous (ms-TRAM) flap breast reconstructions. The trial is designed as a randomized comparison of standard intraoperative clinical assessment versus clinical assessment supplemented with fluorescence imaging, using a standardized protocol that also includes quantitative analysis. This study represents a critical step toward generating high-level evidence for the role of NIRF imaging in reducing complications after reconstructive flap surgery.

In the last part of this thesis, **Chapter 7**, perfusion patterns of vital tissues are quantified using NIRF imaging, with the aim of establishing a reference perfusion curve for a specific tissue. Measurements were performed across ten tissue types, derived from five study cohorts in three hospitals. Each tissue type demonstrated a characteristic perfusion pattern, with normalized curves displaying only limited variation within tissue groups despite differences in imaging protocols. At the same time, comparable patterns were observed between most tissue types. These findings provide a first step toward standardized benchmarks, enabling broader comparability and future definition of clinically relevant cut-off values for adequate perfusion.

Lastly, **Chapter 8** discusses the broader implications and future perspectives of quantitative NIRF imaging. It highlights that while feasibility and clinical applicability have been demonstrated across surgical domains, challenges remain regarding parameter selection, standardization of protocols, and clinical validation in larger outcome-driven trials. Future integration will depend on automated analysis, multicenter collaboration, and possibly complementary imaging techniques, with the ultimate goal of evolving quantitative NIRF imaging into a clinically integrated tool that reduces perfusion-related complications and improves surgical outcomes.

Discussion

The assessment of tissue perfusion is a pivotal element of surgical decision-making, yet current methods remain limited in their reliability and standardization (1-5). Near-infrared fluorescence (NIRF) imaging with indocyanine green (ICG) is an established technique for assessing tissue perfusion, yet its interpretation in clinical practice predominantly relies on subjective evaluation (6-9). In this thesis, quantitative NIRF imaging with ICG was evaluated across several surgical domains, including peripheral arterial disease, colorectal and oesophageal surgery, free flap procedures, and in the establishment of general reference values for multiple tissues. These studies collectively demonstrate that quantitative analysis is technically feasible, broadly applicable, and provides more consistent and objective information about tissue perfusion compared to visual assessment alone.

Subjective interpretation of fluorescence could be useful in specific situations such as amputation surgery (10). However, in most other surgical contexts without evident ischemic zones or demarcation borders, its reliability is limited and quantitative analysis offers the potential to provide reproducible and objective information (11-13). Among the quantification parameters explored, absolute (intensity-related) parameters repeatedly proved to be highly variable due to strong dependence on factors such as camera settings, distance, and injected ICG dose (14-17). In contrast, time-based and normalised parameters, such as time-to-maximum intensity (Tmax) and normalized maximum slope (norm slope), demonstrated robustness and association with clinical outcomes, as also demonstrated in this thesis. Furthermore, existing literature has focussed on quantitative parameters related to the inflow phase, which seem to correlate best with clinical outcomes (12, 13). This thesis consistently included outflow analysis, but its clinical value remains unclear and should be addressed in larger cohorts (15, 18, 19)

Normalization may improve comparability of perfusion measurements, potentially even when different camera systems or imaging protocols are used (14, 15). This is supported by the reference curve study in this thesis, where normalized data produced with variations of imaging protocols showed very limited variation. This finding suggests that normalization can reduce methodological bias and possibly facilitate multicenter comparison. Beyond technical comparability, clinical interpretation requires a reference to distinguish adequately perfused from poorly perfused tissue. Internal references, such as adjacent well-perfused colonic tissue, can provide an optimal reference (20, 21). Though, internal references are not always available during measurements, for example in patients with ischemic limbs. External reference curves based on healthy tissue therefore offer an important alternative, although their applicability may be influenced by inter-patient differences including hemodynamic variability. Larger cohorts are needed to quantify and if necessary correct for these effects.

In settings where intra-arterial access is already established, such as vascular procedures, intra-arterial ICG administration could be considered to minimize systemic hemodynamic influences (22). Importantly, this thesis demonstrated that establishing such external references is both feasible and clinically relevant, providing a first step toward broader standardization.

The studies of this thesis have several common limitations, including relatively small cohorts and outcomes that were often based on indirect measures rather than conclusive clinical endpoints. Nevertheless, the studies in this thesis were primarily designed to demonstrate feasibility and applicability of quantification across surgical domains, and not to determine threshold values or prove final clinical impact. The favourable outcomes of the studies indicate that the next step will be larger, outcome-driven trials, as already exemplified by the study protocol of the multicenter DIEP flap RCT included in this thesis. Of note, quantification was performed postoperatively, as results had no direct clinical consequences. Nevertheless, intraoperative quantification is technically feasible and could already be applied in practice, indicating that implementation should not be hindered by this aspect (21, 23). A further strength of this thesis is the multicenter design of most studies, which demonstrates that standardized data collection across institutions is achievable and provides a solid foundation for future large-scale studies.

The studies in this thesis illustrate how quantitative ICG NIR fluorescence may translate into clinical benefit for multiple surgical applications. In peripheral arterial disease, quantitative analysis could facilitate early detection of perfusion alterations, enabling more accurate prediction of postinterventional outcome and allow for faster transition to additional treatment when needed. Moreover, real-time assessment of perfusion during endovascular procedures has the potential to directly inform whether additional treatment is required. The angiosome study additionally demonstrated that perfusion does not consistently follow anatomical arterial borders, underlining the need for objective imaging rather than relying on theoretical models. In colorectal and oesophageal surgery, more reliable identification of poorly perfused anastomotic sites could be provided, thereby reducing the risk of leakage. In reconstructive surgery, malperfused skin zones could be objectively identified, hereby guiding intraoperative adjustments to lower the risk of fat necrosis and all related negative patient outcomes. Finally, the development of reference curves demonstrated that standardized benchmarks can be established, paving the way toward uniform cut-off values and wider clinical implementation.

As demonstrated in this thesis, the clinical potential of quantitative ICG fluorescence imaging is substantial, but it should be emphasized that perfusion is only one of several factors contributing to postoperative complications (24-27). This means that while objective perfusion assessment can play a major role, it cannot prevent all adverse outcomes.

Surgeons' clinical judgment therefore remains essential, and ICG analysis should be seen as complementary rather than substitutive. Other modalities such as hyperspectral and laser speckle imaging may provide additional information, or even be of superior value in certain circumstances (28-31).

A further consideration is that all studies in this thesis were performed with open camera systems. Such an approach will remain indispensable in settings such as vascular surgery where skin perfusion is evaluated. At the same time, laparoscopic and robotic procedures are common, for instance in gastrointestinal surgery, during which standardized camera distance and angle are barely achievable (32, 33). Although highly relevant, the applicability of quantitative fluorescence imaging in minimally invasive procedures was beyond the scope of this thesis.

Future perspectives

The most important perspective of this thesis is how quantitative ICG fluorescence imaging can develop into a clinically integrated tool in the near future. A key requirement will be automation of fluorescence analysis. Current manual workflows are time-consuming and inconsistent for routine use. Automated solutions that provide real-time curve evaluation, perfusion heatmaps, and threshold-based alerts could transform the technique from a research tool into an intuitive aid for surgeons. Artificial intelligence may play a central role in this development. By detecting complex perfusion patterns and integrating multiple parameters simultaneously, AI-driven models could enhance accuracy and consistency beyond what conventional analyses can achieve. In addition, AI could assist in defining clinically relevant cut-off values, an important need that would enable objective and actionable intraoperative decision-making.

Another promising direction is the exploration and possibly combination of imaging modalities. Techniques such as hyperspectral imaging (HSI), laser speckle imaging (LSCI) and optical coherence tomography (OCT) each capture different aspects of tissue perfusion and vitality. Their integration with ICG fluorescence may provide a more comprehensive assessment of tissue health. Exploring when and how to combine these approaches will be an important focus for future research.

Extending quantitative fluorescence imaging to minimally invasive procedures will be essential for broad clinical adoption, as these procedures are increasingly common. Time-related and normalized parameters may provide the robustness needed to overcome variability in camera distance and angle during laparoscopy and robotic surgery. Moreover, the integration of fluorescence modes into most modern minimally invasive platforms offers an opportunity for seamless clinical implementation once validated analysis tools become available.

Finally, significant progress will require large, outcome-driven clinical trials to demonstrate that objective perfusion assessment can reduce complications related to malperfusion. Such studies will demand broad international and multicenter collaboration, not only among surgeons but also with data scientists and industry partners to harmonize protocols, develop user-friendly software, and scale implementation. The studies in this thesis already demonstrate that multicenter data collection and standardization are achievable, providing a foundation for this next phase.

Ultimately, quantitative ICG fluorescence imaging should evolve from an experimental tool into an integrated element of surgical decision-making. If these steps are realized, the technique has the potential to reduce complication rates, improve patient safety, and advance surgical precision across multiple domains.

References

1. Misra S, Shishehbor MH, Takahashi EA, Aronow HD, Brewster LP, Bunte MC, et al. Perfusion Assessment in Critical Limb Ischemia: Principles for Understanding and the Development of Evidence and Evaluation of Devices: A Scientific Statement From the American Heart Association. Circulation. 2019 Sep 17;140(12):e657–72.
2. Karliczek A, Harlaar NJ, Zeebregts CJ, Wiggers T, Baas PC, Van Dam GM. Surgeons lack predictive accuracy for anastomotic leakage in gastrointestinal surgery. Int J Colorectal Dis. 2009 May;24(5):569–76.
3. Ryu HS, Lim SB, Choi ET, Song I, Lee JL, Kim CW, et al. Intraoperative perfusion assessment of the proximal colon by a visual grading system for safe anastomosis after resection in left-sided colorectal cancer patients. Sci Rep. 2021 Feb 2;11(1):2746.
4. Smit JM, Zeebregts CJ, Acosta R, Werker PMN. Advancements in Free Flap Monitoring in the Last Decade: A Critical Review: Plastic and Reconstructive Surgery. 2010 Jan;125(1):177–85.
5. Van Den Hoven P, Goncalves LN, Quax PHA, Van Rijswijk CSP, Van Schaik J, Schepers A, et al. Perfusion Patterns in Patients with Chronic Limb-Threatening Ischemia versus Control Patients Using Near-Infrared Fluorescence Imaging with Indocyanine Green. Biomedicines. 2021 Oct 9;9(10):1417.
6. Hardy NP, Joosten JJ, Dalli J, Hompes R, Cahill RA, Van Berge Henegouwen MI. Evaluation of inter-user variability in indocyanine green fluorescence angiography to assess gastric conduit perfusion in esophageal cancer surgery. Diseases of the Esophagus. 2022 Nov 15;35(11):doac016.
7. Armstrong G, Croft J, Corrigan N, Brown JM, Goh V, Quirke P, et al. IntAct: intra-operative fluorescence angiography to prevent anastomotic leak in rectal cancer surgery: a randomized controlled trial. Colorectal Dis. 2018 Aug;20(8):O226–34.
8. Faber RA, Meijer RPJ, Droogh DHM, Jongbloed JJ, Bijlstra OD, Boersma F, et al. Indocyanine green near-infrared fluorescence bowel perfusion assessment to prevent anastomotic leakage in minimally invasive colorectal surgery (AVOID): a multicentre, randomised, controlled, phase 3 trial. The Lancet Gastroenterology & Hepatology. 2024 Oct;9(10):924–34.
9. Watanabe J, Takemasa I, Kotake M, Noura S, Kimura K, Suwa H, et al. Blood Perfusion Assessment by Indocyanine Green Fluorescence Imaging for Minimally Invasive Rectal Cancer Surgery (EssentiAL trial): A Randomized Clinical Trial. Annals of Surgery. 2023 Oct;278(4):e688–94.
10. Van Den Hoven P, Van Den Berg SD, Van Der Valk JP, Van Der Krogt H, Van Doorn LP, Van De Bogt KEA, et al. Assessment of Tissue Viability Following Amputation Surgery Using Near-Infrared Fluorescence Imaging With Indocyanine Green. Annals of Vascular Surgery. 2022 Jan;78:281–7.
11. Van Den Hoven P, Osterkamp J, Nerup N, Svendsen MBS, Vahrmeijer A, Van Der Vorst JR, et al. Quantitative perfusion assessment using indocyanine green during surgery — current applications and recommendations for future use. Langenbecks Arch Surg. 2023 Jan 26;408(1):67.
12. Lütken CD, Achiam MP, Osterkamp J, Svendsen MB, Nerup N. Quantification of fluorescence angiography: Toward a reliable intraoperative assessment of tissue perfusion - A narrative review. Langenbecks Arch Surg. 2021 Mar;406(2):251–9.
13. Goncalves LN, Van Den Hoven P, Van Schaik J, Leeuwenburgh L, Hendricks CHF, Verduijn PS, et al. Perfusion Parameters in Near-Infrared Fluorescence Imaging with Indocyanine Green: A Systematic Review of the Literature. Life. 2021 May 11;11(5):433.
14. Van Den Hoven P, Tange F, Van Der Valk J, Nerup N, Putter H, Van Rijswijk C, et al. Normalization of Time-Intensity Curves for Quantification of Foot Perfusion Using Near-Infrared Fluorescence Imaging With Indocyanine Green. J Endovasc Ther. 2023 Jun;30(3):364–71.
15. Lütken CD, Achiam MP, Svendsen MB, Boni L, Nerup N. Optimizing quantitative fluorescence angiography for visceral perfusion assessment. Surg Endosc. 2020 Dec;34(12):5223–33.
16. Slooter MD, Mansvelders MSE, Bloemen PR, Gisbertz SS, Bemelman WA, Tanis PJ, et al. Defining indocyanine green fluorescence to assess anastomotic perfusion during gastrointestinal surgery: systematic review. BJS Open. 2021 Mar 5;5(2):zraa074.

17. Noltes ME, Metman MJH, Heeman W, Rotstein L, Van Ginhoven TM, Vriens MR, et al. A Novel and Generic Workflow of Indocyanine Green Perfusion Assessment Integrating Standardization and Quantification Toward Clinical Implementation. Annals of Surgery. 2021 Dec;274(6):e659–63.

18. Van Den Hoven P, S Weller F, Van De Bent M, Goncalves LN, Ruig M, D Van Den Berg S, et al. Near-infrared fluorescence imaging with indocyanine green for quantification of changes in tissue perfusion following revascularization. Vascular. 2022 Oct;30(5):867–73.

19. Joosten JJ, Slooter MD, Van Den Elzen RM, Bloemen PR, Gisbertz SS, Eshuis WJ, et al. Perfusion assessment by fluorescence time curves in esophagectomy with gastric conduit reconstruction: a prospective clinical study. Surg Endosc. 2023 Aug;37(8):6343–52.

20. Meijer RPJ, van Manen L, Hartgrink HH, Burggraaf J, Gioux S, Vahrmeijer AL, et al. Quantitative dynamic near-infrared fluorescence imaging using indocyanine green for analysis of bowel perfusion after mesenteric resection. J Biomed Opt. 2021 Jun;26(6):060501.

21. Nerup N, Svendsen MBS, Svendsen LB, Achiam MP. Feasibility and usability of real-time intraoperative quantitative fluorescent-guided perfusion assessment during resection of gastroesophageal junction cancer. Langenbecks Arch Surg. 2020 Mar;405(2):215–22.

22. Igari K, Kudo T, Uchiyama H, Toyofuku T, Inoue Y. Intraarterial Injection of Indocyanine Green for Evaluation of Peripheral Blood Circulation in Patients with Peripheral Arterial Disease. Annals of Vascular Surgery. 2014 Jul;28(5):1280–5.

23. Tang Y, Gitajn IL, Cao X, Han X, Elliott JT, Yu X, et al. Automated motion artifact correction for dynamic contrast-enhanced fluorescence imaging during open orthopedic surgery. Proc SPIE Int Soc Opt Eng. 2023;12361:1236104.

24. He J, He M, Tang JH, Wang XH. Anastomotic leak risk factors following colon cancer resection: a systematic review and meta-analysis. Langenbecks Arch Surg. 2023 Jun 29;408(1):252.

25. Watanabe M, Takahashi N, Tamura M, Terayama M, Kuriyama K, Okamura A, et al. Gastric conduit reconstruction after esophagectomy. Diseases of the Esophagus. 2024 Oct 2;37(10):doae045.

26. Mills JL, Conte MS, Armstrong DG, Pomposelli FB, Schanzer A, Sidawy AN, et al. The Society for Vascular Surgery Lower Extremity Threatened Limb Classification System: Risk stratification based on Wound, Ischemia, and foot Infection (WIfI). Journal of Vascular Surgery. 2014 Jan;59(1):220-234.e2.

27. Khansa I, Momoh AO, Patel PP, Nguyen JT, Miller MJ, Lee BT. Fat Necrosis in Autologous Abdomen-Based Breast Reconstruction: A Systematic Review. Plastic and Reconstructive Surgery. 2013 Mar;131(3):443–52.

28. Jansen-Winkeln B, Germann I, Köhler H, Mehdorn M, Maktabi M, Sucher R, et al. Comparison of hyperspectral imaging and fluorescence angiography for the determination of the transection margin in colorectal resections—a comparative study. Int J Colorectal Dis. 2021 Feb;36(2):283–91.

29. Studier-Fischer A, Schwab FM, Rees M, Seidlitz S, Sellner J, Özdemir B, et al. ICG-augmented hyperspectral imaging for visualization of intestinal perfusion compared to conventional ICG fluorescence imaging: an experimental study. International Journal of Surgery. 2023 Dec;109(12):3883–95.

30. Skinner GC, Liu YZ, Harzman AE, Husain SG, Gasior AC, Cunningham LA, et al. Clinical Utility of Laser Speckle Contrast Imaging and Real-Time Quantification of Bowel Perfusion in Minimally Invasive Left-Sided Colorectal Resections. Diseases of the Colon & Rectum. 2024 Jun;67(6):850–9.

31. Liu YZ, Shah SK, Sanders CM, Nwaiwu CA, Dechert AF, Mehrotra S, et al. Utility and usability of laser speckle contrast imaging (LSCI) for displaying real-time tissue perfusion/blood flow in robot-assisted surgery (RAS): comparison to indocyanine green (ICG) and use in laparoscopic surgery. Surg Endosc. 2023 Jun;37(6):4803–11.

32. Kong W, Wang B, Niu Q, Lin C, Fan D. Quantitative evaluation of anastomotic perfusion during colorectal surgery via indocyanine green fluorescence angiography: a narrative review. Ann Transl Med. 2022 Dec;10(24):1402–1402.

33. Ahn H, Son GM, Lee IY, Park S, Kim NS, Baek K. Optimization of indocyanine green angiography for colon perfusion during laparoscopic colorectal surgery. Colorectal Disease. 2021 Jul;23(7):1848–59.