review

Laser speckle contrast imaging of perfusion in oncological clinical applications: a literature review

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Radiol Oncol 2024; 58(3): 326-334.

Received 16 July 2024
Accepted 26 July 2024

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Disclosure: No potential conflicts of interest were disclosed.

Background. Laser speckle coherence imaging (LSCI) is an emerging imaging modality that enables noninvasive visualization and assessment of tissue perfusion and microcirculation. In this article, we evaluated LSCI in imaging perfusion in clinical oncology through a systematic review of the literature.

Methods. The inclusion criterion for the literature search in PubMed, Web of Science and Scopus electronic databases was the use of LSCI in clinical oncology, meaning that all animal, phantom, *ex vivo*, experimental, research and development, and purely methodological studies were excluded.

Results. Thirty-six articles met the inclusion criteria. The anatomic locations of the neoplasms in the selected articles were brain (5 articles), breasts (2 articles), endocrine glands (4 articles), skin (12 articles), and the gastrointestinal tract (13 articles).

Conclusions. While LSCI is emerging as an appealing imaging modality, it is crucial for more clinical sites to initiate clinical trials. A lack of standardized protocols and interpretation guidelines are posing the most significant challenge.

Key words: laser speckle contrast imaging (LSCI); oncology; perfusion; blood flow

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Introduction

In the cancer research and treatment, the assessment of tissue perfusion and microcirculation plays a pivotal role in understanding tumor physiology, monitoring treatment responses, and determining surgical outcomes. Among the advanced visualization systems, fluorescence angiography utilizing indocyanine green (FA-ICG) has emerged as an objective tool for evaluating intraoperative perfusion.[1-3] Despite its versatility, FA-ICG imaging has limitations: for example, it requires external dye injection, is constrained by pharmacokinetic factors in repeat assessments, and may potentially lead to allergic reactions to the dye.[2] To overcome these shortcomings, novel imaging techniques have been explored for microvascular imaging.

One such modality is laser speckle contrast imaging (LSCI), a non-invasive optical imaging technique based on the unique properties of laser light to visualize blood flow and tissue perfusion in real-time.[4,5] At the core of LSCI lies the phenomenon of capturing the dynamic interference pattern, known as speckle, created when coherent laser light interacts with moving particles such as red blood cells, generating a real-time 2D color

doi: 10.2478/raon-2024-0042

heatmap of blood flow (Figure 1).[6] By analyzing the temporal fluctuations in the speckle pattern, LSCI can quantitatively assess blood flow velocity, perfusion dynamics, and tissue microcirculation with high spatial and temporal resolution.

LSCI is a versatile modality with its applicability ranging from material science[7] to notable applications in medical therapeutic segments.[8] LSCI has aided, among others, in studying retinal blood flow[9], cardiovascular diseases[10,11] and organ perfusion[6,12], while demonstrating potential as a valuable tool for assessing burns[13-15] and wound healing processes[16-18], and monitoring perfusion during reconstructive surgery[19] and neurosurgery.[20-26] The value of LSCI in quantifying blood flow dynamics within clinical oncology remains unclear, and to that end, we systematically reviewed the literature with a specific focus on studies in which LSCI was conducted on patients in a clinical oncology setting.

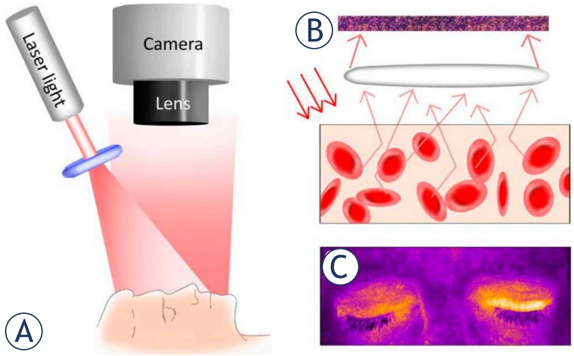

FIGURE 1. Schematic representation of the laser speckle contrast imaging (LSCI) method. **(A)** The technique relies on the interference of light backscattered from moving particles, creating distinct dark and bright areas (speckle pattern) captured by a camera. **(B)** Variations in the speckle pattern are predominantly driven by the movement of red blood cells, enabling interpretation as perfusion. **(C)** Analysis of speckle-pattern variations yields an image displayed on the monitor, where white and yellow depict areas with high perfusion, contrasting with darker areas indicating lower perfusion areas. Taken from Berggren *et al.* [19] and reprinted with permission from the publisher.

Methods

Authors conducted jointly—to minimize potential bias—a comprehensive literature search on April 16, 2024, through PubMed, Web of Science and Scopus electronic databases using the following search terms: “laser speckle coherence imaging tumors”, “laser speckle coherence imaging cancer”, “laser speckle coherence imaging carcinoma”, “laser speckle coherence imaging anastomosis”, and “laser speckle coherence imaging thyroid”. No restrictions on publication date or language were imposed. The inclusion criterion was the application of LSCI in a clinical oncological setting, meaning that all animal and phantom, *ex vivo*, experimental, research and development, and purely methodological studies were excluded. Special care was taken to remove duplicates across databases and studies; for example, if the study was first published in proceedings and later in a journal, the proceedings article was considered a non-primary publication and therefore excluded. Studies were categorized with respect to the anatomical location of the tumors.

Results

In total, 309 articles were found to be of interest in the PubMed, Web of Science and Scopus databases. After excluding duplicates and applying the exclusion criteria, first considering the title and abstract and then, if necessary, reading the entire article, 36 articles were identified for further analysis. The anatomical locations of tumors in the selected articles were as follows: brain (5 articles), breasts (2 articles), endocrine glands (4 articles), skin (12 articles), and the gastrointestinal (GI) tract (13 articles).

Brain

Parthasarathy *et al.*[21] made a pioneering effort in the evaluation of perfusion in clinical oncology using LSCI. Their pilot study focused on imaging cerebral blood flow either before (1 patient) or after (2 patients) tumor resections, across various cortical regions. The same group continued research on larger patient groups (10 and 8, respectively), demonstrating the feasibility of using LSCI to monitor blood flow during neurosurgery.[22,27] Despite these promising outcomes, their research output ceased after 2017.

Another research group[25] highlighted the potential of LSCI for functional brain mapping during awake craniotomy for tumor removal. They observed a strong correlation between cortical microvascular blood flow, as determined by LSCI, and electrocortical stimulation mapping. Additionally, Ideguchi *et al.*[28] emphasized the capability of LSCI for noninvasive and rapid intraoperative real-time recognition of mass lesion-related

TABLE 1. Included articles reporting the use of laser speckle contrast imaging (LSCI) to quantify perfusion in clinical applications in oncology

Reference	Year of publication	Number of patients	Oncologic setting
Brain			
Parthasarathy *et al.*[21]	2010	3	Tumor resection
Richards *et al.*[22]	2014	10	Tumor resection
Richards *et al.*[27]	2017	8	Tumor resection
Klijn *et al.*[25]	2013	8	Tumor resection
Ideguchi *et al.*[28]	2017	12	Tumor resection
Breasts			
Tesselaar *et al.*[29]	2017	15	Adjuvant radiotherapy for stage I-II breast cancer
Zötterman *et al.*[30]	2020	23	Deep inferior epigastric artery perforator (DIEP) flap surgery
Endocrine glands			
de Paula *et al.*[31]	2021	42	Non-functioning adrenal incidentaloma
Mannoh *et al.*[32]	2017	28	Thyroidectomy/parathyroidectomy
Mannoh *et al.*[33]	2021	72	Thyroidectomy
Mannoh *et al.*[34]	2023	21	Thyroidectomy/parathyroidectomy
Skin			
Tchvialeva *et al*·[3]5	2012	214 lesions	Malignant melanoma, squamous cell carcinoma, basal cell carcinoma, melanocytic nevus, seborrheic keratosis
Reyal *et al.*[36]	2012	12	Basal cell carcinoma
Zhang *et al.*[37]	2019	12 (total 143)	Facial nerve palsy due to nerve tumor (also including other etiology)
Zieger *et al.*[38]	2021	9	Basal cell carcinoma
Tenland *et al.*[39]	2019	13	Oculoplastic reconstructive surgery (tarsoconjunctival flaps)
Berggren *et al.*[40]	2019	9	Oculoplastic reconstructive surgery (tarsoconjunctival flaps)
Tenland *et al.*[41]	2021	12	Oculoplastic reconstructive surgery after squamous cell carcinoma, basal cell carcinoma, and intradermal nevus
Berggren *et al.*[42]	2021	7	Oculoplastic reconstructive surgery after squamous cell carcinoma and basal cell carcinoma
Berggren *et al.*[43]	2021	7	Oculoplastic reconstructive surgery after squamous cell carcinoma and basal cell carcinoma
Berggren *et al.*[44]	2021	1	Oculoplastic reconstructive surgery
Berggren *et al.*[45]	2022	7	Oculoplastic reconstructive surgery after squamous cell carcinoma and basal cell carcinoma
Stridh *et al.*[46]	2024	1	Cutaneous angio-sarcoma
Gastrointestinal tract (open surgical setting)			
Eriksson *et al.*[47]	2014	10	Liver resection
Milstein *et al.*[48]	2016	11	Esophagectomy
Ambrus *et al.*[49]	2017	45	Esophagectomy
Ambrus *et al.* [50]	2017	25	Ivor-Lewis esophagectomy
Di Maria *et al.*[51]	2017	2	Colorectal resection
Jansen *et al.*[52]	2018	26	Esophagectomy
Kojima *et al.*[53]	2019	8	Colorectal resection
Kaneko *et al.*[54]	2020	36	Colorectal resection (34 due to colorectal carcinoma)
Gastrointestinal tract (laparoscopic/ thoracoscopic setting)			
Heeman *et al.*[55]	2019	10	Colorectal resection
Kojima *et al.*[56]	2020	27	Colorectal resection
Slooter *et al.*[57]	2020	24	Esophagectomy
Heeman *et al.*[58]	2023	67	Hemicolectomy and sigmoid resection
Nwaiwu *et al.*[59]	2023	40	Colectomy, also non-oncological interventions (Roux-en-Y gastric bypass and sleeve gastrectomy)

vasculature, which could be crucial in mitigating ischemic complications and complementing neurophysiological monitoring.

Breasts

Tesselaar *et al.*[29] conducted a study exploring the relationship between radiation exposure and changes in microvascular perfusion in 15 women undergoing adjuvant radiation therapy for stage I-II breast cancer. Their findings suggested that LSCI holds promise as a useful tool for objectively assessing radiation-induced microvascular changes in the skin, even before visible changes occur, thereby aiding in the earlier prediction of potential severe reactions.

In another prospective clinical pilot study conducted across two centers[30], LSCI was employed in 23 women undergoing primary, secondary, or tertiary deep inferior epigastric artery perforator (DIEP) procedures, either unilateral or bilateral. Researchers used laser speckle patterns to calculate perfusion values in arbitrary units (PU), reflecting the concentration and mean velocity of red blood cells. Categorizing patients into high (> 30) and low (< 30) PU, they found that all flaps with perfusion < 30 PU immediately after surgery had postoperative complications, necessitating revision in 4 women. These results suggest potential utility of LSCI for early detection of flap necrosis, aiding surgeons in identifying viable parts of the flaps. Traditionally, assessment of flap viability relies on subjective methods like skin color, flap temperature, capillary refill time, and dermal edge bleeding.

Endocrine glands

Endothelial reactivity[60,61] was evaluated by LSCI in patients with mostly benign non-functioning adrenal incidentaloma.[31]. Mannoh *et al.*[32] used LSCI to assess parathyroid viability post-thyroidectomy in 20 patients, achieving an accuracy of 91.5% in distinguishing between well vascularized (n = 32) and compromised (n = 27) parathyroid glands compared to visual assessment by an experienced surgeon. Ability to detect vascular compromise with LSCI was further validated in parathyroidectomies in 8 patients, showing that this technique could identify parathyroid gland devascularization before it became visually apparent to the surgeon. LSCI demonstrated promise as a real-time, contrast-free, objective method to mitigate hypoparathyroidism after thyroid surgery.

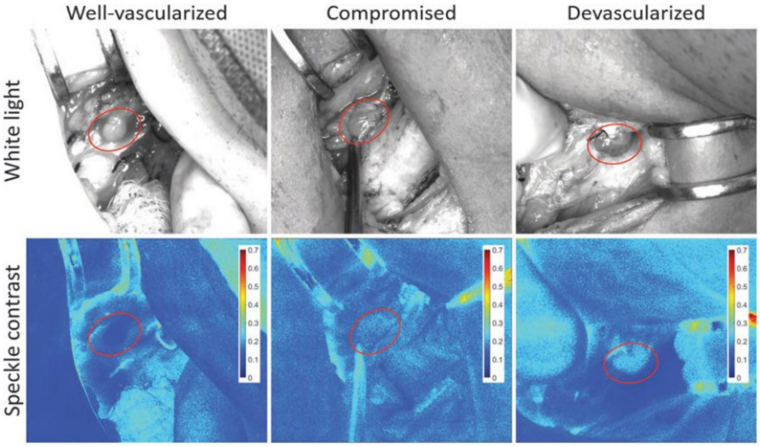

FIGURE 2. Speckle contrast demonstrates lower values for well-vascularized parathyroid glands. Lower speckle contrast values indicate greater blood flow due to more blurring of the speckle pattern, while higher contrast values indicate less blood flow. The top row displays representative white light images, and the bottom row shows speckle contrast images of a well-vascularized (left), a compromised (middle), and a devascularized (right) parathyroid gland, with parathyroid glands marked with ellipses. The corresponding speckle contrast values were 0.11, 0.18, and 0.21, respectively. Taken from Mannoh *et al.* [33] and reprinted with permission from the publisher.

Subsequently, Mannoh *et al.*[33] expanded their research, enrolling 72 patients who underwent thyroidectomy. They established an intraoperative speckle contrast threshold of 0.186 to distinguish between normoparathyroid and hypoparathyroid groups with 87.5% sensitivity and 84.4% specificity. This threshold served as an indicator of adequate parathyroid vascularization, with glands below the value of 0.186 considered adequately perfused (Figure 2).

Additionally, Mannoh *et al.*[34] combined LSCI with ICG angiography in 21 patients undergoing thyroidectomy or parathyroidectomy. While both modalities offered similar information on parathyroid gland blood flow, they suggested advantages of LSCI, including lower costs, non-invasiveness, absence of contraindications, and compatibility with near-infrared autofluorescence (NIRAF) detection, which has recently emerged as a reliable technique for intraoperative parathyroid gland localization or confirmation.[62-64]

Skin

Tchvialeva *et al.*[35] applied LSCI to differentiate among 214 skin lesions, encompassing the three major types of skin cancers (malignant melanoma, squamous cell carcinomas, and basal cell carcinomas – BCCs), and two benign conditions (melanocytic nevus and seborrheic keratoses). In another

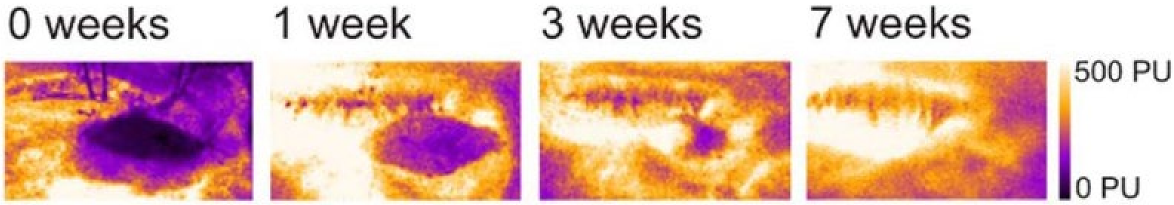

FIGURE 3. Representative examples of laser speckle contrast images, showing the blood perfusion in the free skin grafts, immediately postoperatively (0 weeks), and at follow-up after 1, 3, and 7 weeks. It can be seen that reperfusion occurred simultaneously in the center and periphery of the graft, and that complete reperfusion was achieved after 7 weeks. Taken from Berggren *et al.* [43] and reprinted with permission from the publisher.

early clinical study, LSCI was used to demonstrate that post-occlusive reactive hyperemia could occur in BCC as well.[36] Zhang *et al.*[37] explored differences in facial microvascular perfusion between ipsilateral and contralateral sides in patients with facial nerve palsy (FNP), observing significant decreases on the ipsilateral side, which improved after treatment. In their feasibility study, Zieger *et al.*[38] introduced a compact handheld LSCI device, affirming its reliability in assessing BCC.

In oculoplastics, Tenland *et al.*[39] and Berggren *et al.*[40] conducted studies using LSCI to monitor perfusion in patients with lower eyelid defects after post-tumor surgery large enough to require a tarsoconjunctival graft. Building on their initial work, the group continued research of employing LSCI in various oculoplastic reconstructive surgery procedures. First, Tenland *et al.*[41] monitored perfusion using LSCI in a study in which free bilamellar eyelid grafts appeared to be an excellent alternative to the tarsoconjunctival flap procedure in the reconstruction of both upper and lower eyelid defects. Next, Berggren *et al.*[42] noted rapid revascularization of H-plasty procedure flaps within a week postoperatively, attributing it to the pre-existing vascular network of the flap pedicle, rather than significant angiogenesis. In another study, Berggren *et al.*[43] demonstrated complete reperfusion of skin grafts in the periorbital area after 7 weeks (Figure 3). Berggren *et al.*[44] also presented a case illustrating nearly complete restoration of reperfusion in a rotational full-thickness lower eyelid flap within 5 weeks. Finally, they assessed blood perfusion in glabellar flaps, finding rapid reperfusion.[45] These convincing findings suggest that perioperative LSCI monitoring of perfusion in human periocular flaps and during oculoplastic reconstructive surgery offers an attractive imaging modality for routine clinical use. Not surprisingly, Stridh *et al.*[46] recently conducted a pilot study comprehensively combining LSCI with two other emerging non-invasive medical imaging modalities, hyperspectral imaging [65-67] and photoacoustic imaging[68] to monitor not only blood perfusion but also oxygen saturation and the molecular composition of the tissue.

Gastrointestinal tract (open surgical setting)

The majority of clinical oncology studies with intraoperative LSCI were conducted in an open surgical setting, which we will review first. In an initial pilot clinical study, Eriksson *et al.*[47] assessed liver blood perfusion by occluding the portal vein and hepatic artery in ten consecutive patients undergoing liver resection for colorectal liver metastases. This early effort was followed by Milstein *et al.*[48], who evaluated microvascular blood flow during esophagectomy, affirming that intraoperative LSCI offered a non-contact, non-invasive approach for real-time analysis of potential anastomotic leakage without requiring a contrast medium. This finding was subsequently corroborated by Ambrus *et al.* who first performed gastric microvascular perfusion measurements during esophagectomy in 45 patients[49] and later used LSCI in Ivor-Lewis esophagectomy in 25 patients.[50]

Di Maria *et al.*[51] explored the feasibility of LSCI in 2 patients undergoing colorectal surgery, while Jansen *et al.*[52] investigated the impact of thoracic epidural anesthesia during esophagectomy, once again demonstrating that LSCI could detect subtle changes in gastric microvascular perfusion in real-time. Another group conducted an additional feasibility study of intraoperative LSCI in 8 patients undergoing colorectal surgery.[53] Kaneko *et al.*[54] further expanded on these feasibility studies by enrolling 36 patients undergoing colorectal resection, 34 of whom had colorectal carcinoma, aiming to compare demarcation lines determined by LSCI with transection lines where marginal vessels were divided. They found that 58.3% (21/36) of demarcation lines matched transection lines, with a median distance of 0.0 mm (0.0–12.1 mm) between the demarcation line determined by LSCI and the transection line.

Gastrointestinal tract (laparoscopic/thoracoscopic setting)

Heeman *et al.*[55] reported the first intraabdominal application combining a standard laparoscopic surgical setup with LSCI in 10 patients, enabling imaging of intestinal blood flow during a vascular occlusion test. Their findings were corroborated by Kojima *et al.*[56] in a study involving 27 patients (Figure 4). Slooter *et al.*[57] systematically compared four different emerging optical modalities, highlighting the clinical utility of FA-ICG as the most promising. Recently, Heeman *et al.*[58] tested a commercial LSCI system in the oncological clinical setting, noting that the system was "non-disruptive of the surgical procedure with an average added surgical time of only 2.5 min and no change in surgical equipment". They also observed a potential clinical benefit of the LSCI system, with 17% of operating surgeons altering anastomosis locations based on perfusion assessments. Nwaiwu *et al.*[58] evaluated another commercial intraoperative system combining LSCI and FA-ICG in mostly non-oncological patients, demonstrating that LSCI identified the same perfusion boundaries as FA-ICG, with anastomoses and gastric remnants appearing well perfused.

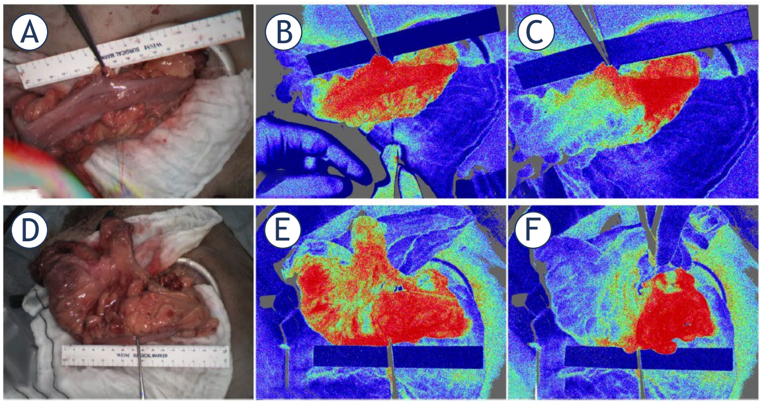

FIGURE 4. Typical laser speckle images in two patients. High-resolution laser speckle contrast imaging (LSCI) can indicate the bowel demarcation line at the point of ligation of the marginal vessels. **(A)** Normal color image before ligating the marginal vessels. **(B)** LSCI image before ligating the marginal vessels. **(C)** LSCI image after ligating the marginal vessels. Taken from Kojima *et al.*[56] and reprinted with permission from the publisher.

Discussion

Based on this literature review, several advantages of LSCI emerge, including its non-invasive and non-contact nature, short acquisition time, high spatial and temporal resolution, low cost of equipment, and simplicity of operation. In the oncological clinical setting, LSCI holds particular promise for assessing skin flap perfusion post-oculoplastic reconstructive surgery and anastomotic perfusion during gastrointestinal reconstruction. While LSCI offers numerous advantages in imaging blood flow dynamics, it is essential to recognize its limitations.

Limited penetration depth

One of the obvious limitations of LSCI in clinical oncology and medical applications, in general, is its restricted penetration depth. LSCI relies on detecting motion contrast generated by moving red blood cells, limiting its applicability to superficial structures. Tumors and lesions located in deeper anatomical locations, such as within organs or soft tissues, may not be adequately visualized due to this limitation, hindering comprehensive evaluation and monitoring of oncological conditions. However, studies like that of Stridh *et al.*[46] demonstrate that PAI as a complementary imaging technique can overcome this limitation. Another possibility to potentially consider is the use of optical clearance techniques[69] to enhance tissue transparency and improve light penetration depth.

Motion artifacts

LSCI is susceptible to motion artifacts, which can arise from either involuntary movement of the subject or vibrations in the imaging setup. These artifacts can lead to image distortions and reduced image quality, compromising the accuracy and reliability of LSCI in clinical oncology. To address this, advanced post-processing algorithms are necessary to improve image quality. Since motion artifacts are well-known sources of artifacts in LSCI, they have been extensively researched. One possibility is to implement motion compensation techniques, such as image stabilization algorithms[70] or gating strategies[71], which can mitigate the effects of motion artifacts in LSCI. By minimizing motion-induced distortions in the speckle pattern, these techniques improve the accuracy and reliability of blood flow measurements.

Inherent speckle noise

The presence of inherent speckle noise in LSCI images can compromise the accuracy and reliability of blood flow measurements, particularly in low-flow regions or under conditions of low contrast. Speckle noise can obscure subtle flow changes and restrict the sensitivity of LSCI in detecting small-scale perfusion variations. Advanced noise reduction algorithms[72] offer a solution by effectively suppressing speckle noise and enhancing the signal-to-noise ratio. These algorithms filter out unwanted noise components while retaining relevant flow information, thereby improving the sensitivity and specificity of LSCI in detecting perfusion changes, even in challenging imaging conditions.

Lack of standardized protocols and interpretation

A significant limitation of LSCI in clinical oncology is the lack of standardized protocols and interpretation guidelines. Varying acquisition settings, image processing algorithms, or interpretation methodologies across different centers can yield inconsistent and non-comparable results. Establishing standardized protocols and guidelines tailored to oncology applications would enhance the accuracy and reproducibility of LSCI findings.

Despite its potential, the clinical integration of LSCI faces obstacles, including the standardization of imaging protocols, validation of its utility in large-scale clinical trials, and integration into existing surgical workflows. Addressing these limitations requires advancements in technology, algorithm refinement, and increased participation of clinical sites in conducting trials. Overcoming these challenges is essential for realizing the full potential of LSCI in clinical oncology; it is worth noting that other biomedical optical imaging techniques [65-67,73-80] are likely to encounter similar challenges in the future.

Acknowledgment

Authors wish to thank Matija Milanič for careful reading of the manuscript and his help with literature search.

This work was financially supported by the state budget by the Slovenian Research Agency, research grant no. J3-3083 and research programs no. P3-0003, P3-0307, P1-0389, P2-0348, and N1-0283.

References

1. Li H, Xie X, Du F, Zhu X, RenH, Ye C, et al. A narrative review of intraoperative use of indocyanine green fluorescence imaging in gastrointestinal cancer: situation and future directions. *J Gastrointest Oncol* 2023; **14:** 1095-113. doi: 10.21037/jgo-23-230
2. Uppal JS, Meng E, Caycedo-Marulanda A. Current applications of indocyanine green fluorescence in colorectal surgery: a narrative review. *Ann Laparosc Endosc Surg* 2023; **8:** 18-18. doi: 10.21037/ales-22-84
3. Iwamoto M, Ueda K, Kawamura J. A narrative review of the usefulness of indocyanine green fluorescence angiography for perfusion assessment in colorectal surgery. *Cancers* 2022; **14:** 5623. doi: 10.3390/cancers14225623
4. Briers D, Duncan DD, Hirst E, Kirkpatric SJ, Larsson M, Steenberg W, et al. Laser speckle contrast imaging: theoretical and practical limitations. *J Biomed Opt* 2013; **18:** 066018. doi: 10.1117/1.JBO.18.6.066018
5. Briers JD, Richards G, He XW. Capillary blood flow monitoring using laser speckle contrast analysis (LASCA). *J Biomed Opt* 1999; **4:** 164. doi: 10.1117/1.429903
6. Draijer M, Hondebrink E, Van Leeuwen T, Steenbergen W. Review of laser speckle contrast techniques for visualizing tissue perfusion. *Lasers Med Sci* 2009; **24:** 639-51. doi: 10.1007/s10103-008-0626-3
7. Hamed AM, El-Ghandoor H, El-Diasty F, Saudy M. Analysis of speckle images to assess surface roughness. *Optics & Laser Technology* 2004; **36:** 249-53. doi: 10.1016/j.optlastec.2003.09.005
8. Heeman W, Steenbergen W, Van Dam GM, Boerma EC. Clinical applications of laser speckle contrast imaging: a review. *J Biomed Opt* 2019; **24:** 1. doi: 10.1117/1.JBO.24.8.080901
9. Cheng H, Yan Y, Duong TQ. Temporal statistical analysis of laser speckle images and its application to retinal blood-flow imaging. *Opt Express* 2008; **16:** 10214. doi: 10.1364/OE.16.010214
10. Hellmann M, Roustit M, Cracowski JL. Skin microvascular endothelial function as a biomarker in cardiovascular diseases? *Pharmacol Rep* 2015; **67:** 803-10. doi: 10.1016/j.pharep.2015.05.008
11. Margouta A, Anyfanti P, Lazaridis A, Nikolaidou B, Mastrogiannis K, Malliora A, et al. Blunted microvascular reactivity in psoriasis patients in the absence of cardiovascular disease, as assessed by laser speckle contrast imaging. *Life* 2022; **12:** 1796. doi: 10.3390/life12111796
12. Gopal JP, Vaz O, Varley R, Spiers H, Goldsworthy MA, Siddagangaiah V, et al. Using laser speckle contrast imaging to quantify perfusion quality in kidney and pancreas grafts on vascular reperfusion: a proof-of-principle study. *Transplant Direct* 2023; **9:** e1472. doi: 10.1097/TXD.0000000000001472
13. Mirdell R, Farnebo S, Sjöberg F, Tesselaar E. Accuracy of laser speckle contrast imaging in the assessment of pediatric scald wounds. *Burns* 2018; **44:** 90-8. doi: 10.1016/j.burns.2017.06.010
14. Mirdell R, Farnebo S, Sjöberg F, Tesselaar E. Interobserver reliability of laser speckle contrast imaging in the assessment of burns. *Burns* 2019; **45:** 1325-35. doi: 10.1016/j.burns.2019.01.011
15. Mirdell R, Farnebo S, Sjöberg F, Tesselaar E. Using blood flow pulsatility to improve the accuracy of laser speckle contrast imaging in the assessment of burns. *Burns* 2020; **46:** 1398-406. doi: 10.1016/j.burns.2020.03.008
16. Rege A, Thakor NV, Rhie K, Pathak AP. In vivo laser speckle imaging reveals microvascular remodeling and hemodynamic changes during wound healing angiogenesis. *Angiogenesis* 2012; **15:** 87-98. doi: 10.1007/s10456-011-9245-x
17. Zheng KJ, Middelkoop E, Stoop M, Van Zuijlen PPM, Pijpe A. Validity of laser speckle contrast imaging for the prediction of burn wound healing potential. *Burns* 2022; **48:** 319-27. doi: 10.1016/j.burns.2021.04.028
18. Mirdell R, Iredahl F, Sjöberg F, Farnebo S, Tesselaar E. Microvascular blood flow in scalds in children and its relation to duration of wound healing: a study using laser speckle contrast imaging. *Burns* 2016; **42:** 648-54. doi: 10.1016/j.burns.2015.12.005
19. Berggren JV, Stridh M, Malmsjö M. Perfusion monitoring during oculoplastic reconstructive surgery: a comprehensive review. *Ophthalmic Plast Reconstr Surg* 2022; **38:** 522-34. doi: 10.1097/IOP.0000000000002114

20. Hecht N, Woitzik J, König S, Horn P, Vajkoczy P. Laser speckle imaging allows real-time intraoperative blood flow assessment during neurosurgical procedures. *J Cereb Blood Flow Metab* 2013; **33:** 1000-7. doi: 10.1038/jcbfm.2013.42

21. Parthasarathy AB, Weber EL, Richards LM, Fox DJ, Dunn AK. Laser speckle contrast imaging of cerebral blood flow in humans during neurosurgery: a pilot clinical study. *J Biomed Opt* 2010; **15:** 066030. doi: 10.1117/1.3526368

22. Richards LM, Towle EL, Fox DJ, Dunn AK. Intraoperative laser speckle contrast imaging with retrospective motion correction for quantitative assessment of cerebral blood flow. *Neurophoton* 2014; **1:** 1. doi: 10.1117/1.NPh.1.1.015006

23. Woitzik J, Hecht N, Pinczolits A, Sandow N, Major S, Winkler MKL, et al. Propagation of cortical spreading depolarization in the human cortex after malignant stroke. *Neurology* 2013; **80:** 1095-102. doi: 10.1212/WNL.0b013e3182886932

24. Hecht N, Müller MM, Sandow N, Pinczolits A, Vajkoczy P, Woitzik J. Infarct prediction by intraoperative laser speckle imaging in patients with malignant hemispheric stroke. *J Cereb Blood Flow Metab* 2016; **36:** 1022-32. doi: 10.1177/0271678X15612487

25. Klijn E, Hulscher HC, Balvers RK, Holland WPJ, Bakker J, Vincent ALPE, et al. Laser speckle imaging identification of increases in cortical microcirculatory blood flow induced by motor activity during awake craniotomy: clinical article. *J Neurosurg* 2013; **118:** 280-86. doi: 10.3171/2012.10.JNS1219

26. Konovalov A, Gadzhiagaev V, Grebenev F, Stavtsev D, Piavchenko G, Gerasimenko A, et al. Laser speckle contrast imaging in neurosurgery: a systematic review. *World Neurosurg* 2023; **171:** 35-40. doi: 10.1016/j.wneu.2022.12.048

27. Richards LM, Kazmi SS, Olin KE, Waldron JS, Fox DJ, Dunn AK. Intraoperative multi-exposure speckle imaging of cerebral blood flow. *J Cereb Blood Flow Metab* 2017; **37:** 3097-109. doi: 10.1177/0271678X16686987

28. Ideguchi M, Kajiwara K, Yoshikawa K, Goto H, Sugimoto K, Inoue T, et al. Avoidance of ischemic complications after resection of a brain lesion based on intraoperative real-time recognition of the vasculature using laser speckle flow imaging. *J Neurosurg* 2017; **126:** 274-80. doi: 10.3171/2016.1.JNS152067

29. Tesselaar E, Flejmer AM, Farnebo S, Dasu A. Changes in skin microcirculation during radiation therapy for breast cancer. *Acta Oncol* 2017; **56:** 1072-80. doi: 10.1080/0284186X.2017.1299220

30. Zötterman J, Opsomer D, Farnebo S, Blondeel P, Monstrey S, Tesselaar E. Intraoperative laser speckle contrast imaging in DIEP breast reconstruction: a prospective case series study. *Plast Reconstr Surg Glob Open* 2020; **8:** e2529. doi: 10.1097/GOX.0000000000002529

31. De Paula MP, Moraes AB, De Souza MDGC, Cavalari EMR, Campbell RC, da Silva Fernandes G, et al. Cortisol level after dexamethasone suppression test in patients with non-functioning adrenal incidentaloma is positively associated with the duration of reactive hyperemia response on microvascular bed. *J Endocrinol Invest* 2021; **44:** 609-19. doi: 10.1007/s40618-020-01360-z

32. Mannoh EA, Thomas G, Solórzano CC, Mahadevan-Jansen A. Intraoperative assessment of parathyroid viability using laser speckle contrast imaging. *Sci Rep* 2017; **7:** 14798. doi: 10.1038/s41598-017-14941-5

33. Mannoh EA, Thomas G, Baregamian N, Rohde SL, Solórzano CC, Mahadevan-Jansen A. Assessing intraoperative laser speckle contrast imaging of parathyroid glands in relation to total thyroidectomy patient outcomes. *Thyroid* 2021: **31:** 1558-65. doi: 10.1089/thy.2021.0093

34. Mannoh EA, Baregamian N, Thomas G, Solórzano CC, Mahadevan-Jansen A. Comparing laser speckle contrast imaging and indocyanine green angiography for assessment of parathyroid perfusion. *Sci Rep* 2023; **13:** 17270. doi: 10.1038/s41598-023-42649-2

35. Tchvialeva L, Dhadwal G, Lui H, Kalia S, Zeng H, McLean DI, et al. Polarization speckle imaging as a potential technique for *in vivo* skin cancer detection. *J Biomed Opt* 2012; **18:** 061211. doi: 10.1117/1.JBO.18.6.061211

36. Reyal J, Lebas N, Fourme E, Guihard T, Vilmer C, Masurier PL. Post-occlusive reactive hyperemia in basal cell carcinoma and its potential application to improve the efficacy of solid tumor therapies. *Tohoku J Exp Med* 2012; **227:** 139-47. doi: 10.1620/tjem.227.139

37. Zhang Y, Zhao L, Li J, Wang J, Yu H. Microcirculation evaluation of facial nerve palsy using laser speckle contrast imaging: a prospective study. *Eur Arch Otorhinolaryngol* 2019; **276:** 685-92. doi: 10.1007/s00405-019-05281-3

38. Zieger M, Kaatz M, Springer S, Riesenberg R, Wuttig A, Kanka M, et al. Multi-wavelength, handheld laser speckle imaging for skin evaluation. *Skin Res Technol* 2021; **27:** 486-93. doi: 10.1111/srt.12959

39. Tenland K, Memarzadeh K, Berggren J, Nguyen CD, Dahlstrand U, Hult J, et al. Perfusion monitoring shows minimal blood flow from the flap pedicle to the tarsoconjunctival flap. *Ophthalmic Plast Reconstr Surg* 2019; **35:** 346-9. doi: 10.1097/IOP.0000000000001250

40. Berggren J, Tenland K, Ansson CD, Dahlstrand U, Sheikh R, Hult J, et al. Revascularization of free skin grafts overlying modified hughes tarsoconjunctival flaps monitored using laser-based techniques. *Ophthalmic Plast Reconstr Surg* 2019; **35:** 378-82. doi: 10.1097/IOP.0000000000001286

41. Tenland K, Berggren J, Engelsberg K, Bohman E, Dahlstrand U, Castelo N, et al. Successful free bilamellar eyelid grafts for the repair of upper and lower eyelid defects in patients and laser speckle contrast imaging of revascularization. *Ophthalmic Plast Reconstr Surg* 2021; **37:** 168-72. doi: 10.1097/IOP.0000000000001724

42. Berggren J, Castelo N, Tenland K, Engelsberg K, Dahlstand U, Albinsson J, et al. Revascularization after H-plasty reconstructive surgery in the periorbital region monitored with laser speckle contrast imaging. *Ophthalmic Plast Reconstr Surg* 2021; **37:** 269-73. doi: 10.1097/IOP.0000000000001799

43. Berggren J, Castelo N, Tenland K, Dahlstrand, Engelsberg K, Lindstedt S, et al. Reperfusion of free full-thickness skin grafts in periocular reconstructive surgery monitored using laser speckle contrast imaging. *Ophthalmic Plast Reconstr Surg* 2021; **37:** 324-8. doi: 10.1097/IOP.0000000000001851

44. Berggren JV, Sheikh R, Hult J, Engelsberg K, Malmsjö M. Laser speckle contrast imaging of a rotational full-thickness lower eyelid flap shows satisfactory blood perfusion. *Ophthalmic Plast Reconstr Surg* 2021; **37:** e139-e141. doi: 10.1097/IOP.0000000000001921

45. Berggren JV, Tenland K, Sheikh R, Hult J, Engelsberg K, Lindstedt S, et al. Laser speckle contrast imaging of the blood perfusion in glabellar flaps used to repair medial canthal defects. *Ophthalmic Plast Reconstr Surg* 2022; **38:** 274-9. doi: 10.1097/IOP.0000000000002082

46. Stridh M, Dahlstrand U, Naumovska M, Engelsberg K, Gesslein B, Sheikh R, et al. Functional and molecular 3D mapping of angiosarcoma tumor using non-invasive laser speckle, hyperspectral, and photoacoustic imaging. *Orbit* 2024; **9:** 1-11. doi: 10.1080/01676830.2024.2331718

47. Eriksson S, Jan N, Gert L, Sturesson C. Laser speckle contrast imaging for intraoperative assessment of liver microcirculation: a clinical pilot study. *Med Devices* 2014; 25: 257-61. doi: 10.2147/MDER.S63393

48. Milstein DMJ, Ince C, Gisbertz SS, Boateng KB, Geerts BF, Hollmann MW, et al. Laser speckle contrast imaging identifies ischemic areas on gastric tube reconstructions following esophagectomy. *Medicine* 2016; **95:** e3875. doi: 10.1097/MD.0000000000003875

49. Ambrus R, Achiam MP, Secher NH, Svendsen MB, Runitz K, Siemsen M, et al. Evaluation of gastric microcirculation by laser speckle contrast imaging during esophagectomy. *J Am Col Surg* 2017; **225:** 395-402. doi: 10.1016/j.jamcollsurg.2017.06.003

50. Ambrus R, Svendsen LB, Secher NH, Runitz K, Frediriksen HJ, Svendsen MBS, et al. A reduced gastric corpus microvascular blood flow during Ivor-Lewis esophagectomy detected by laser speckle contrast imaging technique. *Scand J Gastroenterol* 2017; **52:** 455-61. doi: 10.1080/00365521.2016.1265664

51. Di Maria C, Hainsworth PJ, Allen J. Intraoperative thermal and laser speckle contrast imaging assessment of bowel perfusion in two cases of colorectal resection surgery. In: Ng EY, Etehadtavakol M, editors. *Application of infrared to biomedical sciences*. Series in BioEngineering. Singapore: Springer; 2017. p. 437-49. doi: 10.1007/978-981-10-3147-2_25

52. Jansen SM, De Bruin DM, Van Berge Henegouwen MI, Bloemen PR, Strackee SD, Veelo DP, et al. Effect of ephedrine on gastric conduit perfusion measured by laser speckle contrast imaging after esophagectomy: a prospective in vivo cohort study. *Dis Esophagus* 2018; **1:** 31. doi: 10.1093/dote/doy031

53. Kojima S, Sakamoto T, Nagai Y, Matsui Y, Nambu K, Masamune K. Laser speckle contrast imaging for intraoperative quantitative assessment of intestinal blood perfusion during colorectal surgery: a prospective pilot study. *Surg Innov* 2019; **26:** 293-301. doi: 10.1177/1553350618823426

54. Kaneko T, Funahashi K, Ushigome M, Kagami S, Yoshida K, Koda T, et al. Noninvasive assessment of bowel blood perfusion using intraoperative laser speckle flowgraphy. *Langenbecks Arch Surg* 2020; **405:** 817-26. doi: 10.1007/s00423-020-01933-9

55. Heeman W, Dijkstra K, Hoff C, Koopal S, Pierie JP, Bouma H, et al. Application of laser speckle contrast imaging in laparoscopic surgery. *Biomed Opt Express* 2019; **10:** 2010-9. doi: 10.1364/BOE.10.002010
56. Kojima S, Sakamoto T, Matsui Y, Nambu K, Masamune K. Clinical efficacy of bowel perfusion assessment during laparoscopic colorectal resection using laser speckle contrast imaging: a matched case-control study. *Asian J Endoscop Surgery* 2020; **13:** 329-35. doi: 10.1111/ases.12759
57. Slooter MD, Jansen SMA, Bloemen PR, van den Elzen RM, Wilk LS, van Leeuwen TG, et al. Comparison of optical imaging techniques to quantitatively assess the perfusion of the gastric conduit during oesophagectomy. *Applied Sciences* 2020; **10:** 5522. doi: 10.3390/app10165522
58. Heeman W, Calon J, Van Der Bilt A, Pierie JPEN, Pereboom I, van Dam GM, et al. Dye-free visualisation of intestinal perfusion using laser speckle contrast imaging in laparoscopic surgery: a prospective, observational multi-centre study. *Surg Endosc* 2023; **37:** 9139-46. doi: 10.1007/s00464-023-10493-0
59. Nwaiwu CA, McCulloh CJ, Skinner G, Shah SK, Kim PC, Schwaitzberg SD, et al. Real-time first-in-human comparison of laser speckle contrast imaging and ICG in minimally invasive colorectal & bariatric surgery. *J Gastrointest Surgery* 2023; **27:** 3083-5. doi: 10.1007/s11605-023-05855-x
60. Yataco AR, Corretti MC, Gardner AW, Womack CJ, Katzel LI. Endothelial reactivity and cardiac risk factors in older patients with peripheral arterial disease. *Am J Cardiol* 1999; **83:** 754-8. doi: 10.1016/S0002-9149(98)00984-9
61. Souza EG, De Lorenzo A, Huguenin G, Oliveira GMM, Tibiriçá E. Impairment of systemic microvascular endothelial and smooth muscle function in individuals with early-onset coronary artery disease: studies with laser speckle contrast imaging. *Coron Artery Dis* 2014; **25:** 23-28. doi: 10.1097/MCA.0000000000000055
62. Paras C, Keller M, White L, Phay J, Mahadevan-Jansen A. Near-infrared autofluorescence for the detection of parathyroid glands. *J Biomed Opt* 2011; **16:** 067012. doi: 10.1117/1.3583571
63. Benmiloud F, Godiris-Petit G, Gras R, Gillot JC, Turrin N, Penarada G, et al. Association of autofluorescence-based detection of the parathyroid glands during total thyroidectomy with postoperative hypocalcemia risk: results of the PARAFLUO multicenter randomized clinical trial. *JAMA Surg* 2020; **155:** 106. doi: 10.1001/jamasurg.2019.4613
64. Dip F, Falco J, Verna S, Prunello M, Loccisano M, Quadri P, et al. Randomized controlled trial comparing white light with near-infrared autofluorescence for parathyroid gland identification during total thyroidectomy. *J Am Coll Surg* 2019; **228:** 744-51. doi: 10.1016/j.jamcollsurg.2018.12.044
65. Stergar J, Hren R, Milanič M. Design and validation of a custom-made laboratory hyperspectral imaging system for biomedical applications using a broadband LED light source. *Sensors* 2022; **22:** 6274. doi: 10.3390/s22166274
66. Hren R, Sersa G, Simoncic U, Milanic M. Imaging perfusion changes in oncological clinical applications by hyperspectral imaging: a literature review. *Radiol Oncol* 2022; **56:** 420-9. doi: 10.2478/raon-2022-0051
67. Hren R, Stergar J, Simončič U, Serša G, Milanič M. Assessing perfusion changes in clinical oncology applications using hyperspectral imaging. In: Jarm T, Šmerc R, Mahnič-Kalamiza S, editors. *9th European Medical and Biological Engineering Conference*. Portorož, Slovenia; 2024 Jun 9-13. Vol 112. IFMBE Proceedings. Switzerland: Springer Nature; 2024. p. 122-9. doi: 10.1007/978-3-031-61625-9_14
68. Lin L, Wang LV. The emerging role of photoacoustic imaging in clinical oncology. *Nat Rev Clin Oncol* 2022; **19:** 365-84. doi: 10.1038/s41571-022-00615-3
69. Xia Q, Li D, Yu T, Zhu J, Zhu D. In vivo skin optical clearing for improving imaging and light-induced therapy: a review. *J Biomed Opt* 2023; **28:** 060901. doi: 10.1117/1.JBO.28.6.060901
70. Heeman W, Maassen H, Dijkstra K, Calon J, van Goor H, Leuvenik H, et al. Real-time, multi-spectral motion artefact correction and compensation for laser speckle contrast imaging. *Sci Rep* 2022; **12:** 21718. doi: 10.1038/s41598-022-26154-6
71. Gnyawali SC, Blum K, Pal D, Ghatak S, Khanna S, Roy S, et al. Retooling laser speckle contrast analysis algorithm to enhance non-invasive high resolution laser speckle functional imaging of cutaneous microcirculation. *Sci Rep* 2017; **7:** 41048. doi: 10.1038/srep41048
72. Han G, Li D, Wang J, Guo Q, Yuan J, Chen R, et al. Adaptive window space direction laser speckle contrast imaging to improve vascular visualization. *Biomed Opt Express* 2023; **14:** 3086. doi: 10.1364/BOE.488054
73. Hren R, Sersa G, Simoncic U, Milanic M. Imaging microvascular changes in nonocular oncological clinical applications by optical coherence tomography angiography: a literature review. *Radiol Oncol* 2023; **57:** 411-8. doi: 10.2478/raon-2023-0057
74. Stergar J, Hren R, Milanič M. Design and validation of a custom-made hyperspectral microscope imaging system for biomedical applications. *Sensors* 2023; **23:** 2374. doi: 10.3390/s23052374
75. Marin A, Hren R, Milanič M. Pulsed photothermal radiometric depth profiling of bruises by 532 nm and 1064 nm lasers. *Sensors* 2023; **23:** 2196. doi: 10.3390/s23042196
76. Rogelj L, Dolenec R, Tomšič MV, Laister E, Simončič U, Milanič M, et al. Anatomically accurate, high-resolution modeling of the human index finger using in vivo magnetic resonance imaging. *Tomography* 2022; **8:** 2347-59. doi: 10.3390/tomography8050196
77. Milanic M, Hren R, Stergar J, Simoncic U. Monitoring of caffeine consumption effect on skin blood properties by diffuse reflectance spectroscopy. *Physiol Res* 2024; **73:** 47-56. doi: 10.33549/physiolres.935138
78. Marin A, Verdel N, Milanič M, Majaron B. Noninvasive monitoring of dynamical processes in bruised human skin using diffuse reflectance spectroscopy and pulsed photothermal radiometry. *Sensors* 2021; **21:** 302. doi: 10.3390/s21010302
79. Milanic M, Marin A, Stergar J, Verdel N, Majaron B. Monitoring of caffeine consumption effect on skin blood properties by diffuse reflectance spectroscopy. In: Dehghani H, Wabnitz H, editors. *Diffuse optical spectroscopy and imaging VI*. SPIE Proceedings; European Conference on Biomedical Optics 2017. Munich Germany; 25-29 Jun 2017. Paper 1041215. doi: 10.1117/12.2286140
80. Verdel N, Marin A, Milanič M, Majaron B. Physiological and structural characterization of human skin in vivo using combined photothermal radiometry and diffuse reflectance spectroscopy. *Biomed Opt Express* 2019; **10:** 944. doi: 10.1364/BOE.10.000944