Comparison of laser speckle contrast imaging with laser Doppler perfusion imaging for tissue perfusion measurement

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Abstract

Objective: Laser-based tissue perfusion monitoring techniques have been increasingly used in animal and human research to assess blood flow. However, these techniques use arbitrary units, and knowledge about their comparability is scarce. This study aimed to model the relationship between laser speckle contrast imaging (LSCI) and laser Doppler perfusion imaging (LDPI), for measuring tissue perfusion over a wide range of blood flux values.

Methods: Fifteen healthy volunteers (53% female, median age 29 [IQR 22–40] years) were enrolled in this study. We performed iontophoresis with sodium nitroprusside on the forearm to induce regional vasodilation to increase skin blood flux. Besides, a stepwise vascular occlusion was applied on the contralateral upper arm to reduce blood flux. Both techniques were compared using a linear mixed model analysis.

Results: Baseline blood flux values measured by LSCI were 33 ± 6.5 arbitrary unit (AU) (Coefficient of variation [CV] = 20%) and by LDPI 60 ± 11.5 AU (CV = 19%). At the end of the iontophoresis protocol, the regional blood flux increased to 724 ± 412% and 259 ± 87% of baseline measured by LDPI and LSCI, respectively. On the other hand, during the stepwise vascular occlusion test, the blood flux reduced to 212 ± 40% and 412 ± 177% of its baseline at LDPI and LSCI, respectively. A strong correlation was found between the LSCI and LDPI instruments at increased blood flux with respect to baseline skin blood flux; however, the correlation was weak at reduced blood flux with respect to baseline.

Discussion: LSCI and LDPI instruments are highly linear for blood flux higher than baseline skin blood flux; however, the correlation decreased for blood flux lower than baseline. This study’s findings could be a basis for using LSCI in specific patient populations, such as burn care.

Abbreviations: AU, arbitrary unit; CV, coefficient of variation; LBIs, laser-based techniques; LDF, laser doppler flowmetry; LDPI, laser doppler perfusion imaging; LSCI, laser speckle contrast imaging; RBC, red blood cell; μA, microampere.

Goksel Guven and Annemieke Dijkstra contributed equally to this work.

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1  |  INTRODUCTION

The microcirculation consists of arterioles, venules, and capillaries, which regulate red blood cell microvascular blood flow to optimally provide the tissues with oxygen. For the last two decades, laser-based techniques (LBTs) have increasingly been used for assessing microcirculatory perfusion in human and animal research. Combined with a provocation test, LBTs can also be used to estimate microvascular reactivity, whose alteration is considered a risk factor for cardiovascular disease. Skin surface has been one of the most preferred surrogates for monitoring tissue microcirculation with LBTs, due to its easy access.

Fundamentally, laser-based monitoring of microcirculatory perfusion can be achieved by two techniques; laser Doppler imaging and Laser speckle contrast imaging (LSCI). Laser Doppler imaging consists of two distinct techniques: Laser Doppler flowmetry (LDF) and laser Doppler perfusion imaging (LDPI). The LDF continuously assesses the blood flux of small surfaces (<1 mm²) and needs probes to contact the tissue. This technique has low spatial resolution and low reproducibility despite having a high temporal resolution. On the other hand, LDPI is a non-invasive and non-contact technique used to estimate skin microvascular blood flux in a 50 × 50 cm² area. LDPI technique has high spatial resolution and good reproducibility. However, the relatively slow scanning procedure leads to a low temporal resolution, making it of limited value as a technique to estimate microvascular reactivity, which requires a high temporal reactivity. The large size, long scanning time, and limited maneuvering capability disable the LDPI instruments to continuously monitor at the bedside. Therefore, much quicker, maneuverable, and high-quality instruments are necessitated for use in clinical practice.

Laser speckle contrast imaging is another non-invasive and non-contact microvascular imaging technique, which enables continuous and real-time recording of microvascular blood flux of large skin surfaces. It is a relatively novel technique that works on the basis of change in speckle contrast that produces an index of blood flow. Compared to LDPI, LSCI has a higher spatial and temporal resolution and excellent reproducibility. Moreover, it is quicker and has a faster way of signal processing at the bedside, which may overcome the limitations of LDPI. On the other hand, the LSCI is more sensitive to movement artifacts and assesses smaller skin surfaces than the LDPI technique.

There are two main manufacturers developing laser-based instruments; Perimed (Järfalla, Sweden) and Moor (Devon, UK). Each manufacturer uses its specific arbitrary unit (AU) to define the output. However, a lack of uniformity exists in data expression among both techniques. Therefore, the interpretation of the output and interchangeable use of the instruments becomes complicated.

In this study, we aimed to model the relationship between the Perimed LSCI and Moor LDPI instruments over a wide range of blood flux values by applying a pharmacologic provocation test (sodium nitroprusside iontophoresis technique) and stepwise vascular occlusion test to obtain higher and lower blood flux values, respectively. As the secondary aim, we compared arbitrary units of these two different kinds of laser-based instruments and created a standard universal unit model.

2  |  MATERIALS AND METHODS

2.1  |  Study population

Fifteen healthy volunteers were enrolled in the study. Exclusion criteria were as follows: pregnancy, age under 18 years, having active or chronic disease, being under any medication, having an allergy to sodium nitroprusside, and smoking history. Each subject was recruited by social media contact and provided written informed consent before participation. The Medical Research Ethics Committees United (MEC-U) approved this study with the number NL65747.101.18. (Clinical Trials ID, NCT04660162).

2.2  |  Study design

The volunteers were instructed to beware of drinking coffee or eating a meal within 2 h before study initiation. All subjects had at least 20 min of acclimatization period resting in a semi-supine position. In order to equalize the environmental confounders, all the measurements were performed in the same temperature-controlled room (23 ± 1°C), which was intermittently checked with a thermometer. The door was closed to prevent air movement; lights were switched off, and the curtains were all closed to prevent the interplay of light on instruments. A black vacuum pillow was used to hold the arm stable and to limit reflectivity during the study protocol. Vital signs (heart rate, systemic blood pressure, and temperature) were measured and recorded.

2.3  |  Study protocols

As recommended by the manufacturers, the LSCI and LDPI instruments’ heads were placed at a 20 and 75 cm distance to the skin, respectively. Also, the LSCI instrument was placed under the LDPI instrument and horizontally manipulated so as not to interfere with laser light during the measurements. All the volunteers participated in two consecutive protocols, first the iontophoresis protocol and
second the stepwise vascular occlusion protocol. Each phase consisted of a series of measurements triggered with a certain time interval of around 225 s. This interval is required for measuring the blood flux with both LDPI and LSCI devices. The time point of each measurement was defined as measurement occasion. At each time point, one measurement was obtained from each instrument. The instrument that was used first at every time point was alternated between volunteers. If LDPI was used first in one volunteer, LSCI was used first in the next volunteer, and so on.

PeriIont Micropharmacology System (Perimed AB, Järfälla, Sweden) was used for the iontophoresis technique. The inner side of the right forearm was used for the procedure. Once the skin surface was cleaned with alcohol gauze, the drug delivery and the dispersive electrodes (Perimed AB) were attached with an approximately 10–15 cm distance. The protective tape layer on the drug delivery electrode was peeled off and filled with 0.5 ml of a 10 mg/ml sodium nitroprusside solution by using a syringe. The laser measurement area and image acquisition rate were set at 100 cm² (10 cm × 10 cm) and 21 images/s, respectively. Basal skin blood flux was measured with both instruments before starting iontophoresis. After that, the cathodal iontophoresis was applied with a current strength of 200 μA for 1 min. Subsequently, the iontophoresis device was started, and measurements were done alternately between the two instruments for a maximum of 60 min. However, if the blood flux reached the baseline value before the end of the one-hour study period, measurements at the iontophoresis protocol stopped at that time point. An example of the comparison is shown in Figure 1.

For the stepwise vascular occlusion protocol, a pressure cuff was placed on the upper left. Three different circular measurement areas (approximately 20 mm²) spared from visible vessels, pigmentation, and hair were marked with a pen. The average value of baseline blood flux was recorded. After that, a pneumatic cuff was inflated to 50, 80, 110, 140, and 170 mm Hg and held for 30 s during every increase in blood pressure level. The volunteer had 5 min rest between every step to let the blood flux turn to the baseline between each level.

2.4 | Blood flux measurements

2.4.1 | Laser speckle contrast imaging (LSCI)

Perimed PeriCam LSCI (Perimed AB, Järfälla, Sweden) uses a 70 mW laser power and coherent laser light with a wavelength of 785 nm. The laser light penetrates the tissue and hits the mobile red blood cells. A portion of the laser light backscatters in varying amounts and creates speckle contrast depending on red blood cells’ speed and concentration. The change in speckle contrast and decoloration

![FIGURE 1](image-url)
time quantify an output in AUs. The distance between the detector and the skin is recommended to be 10–35 cm by the manufacturer. The size of the scan area changes between 5.9 × 5.9 cm and 23.7 × 23.7 cm depending on the distance of the detector from the skin. The maximum pixel resolution and minimum pixel dimension are 6944 pixels/cm² and 120 nm, respectively.

2.4.2 Laser Doppler perfusion imaging (LDPI)

MoorLDI2-BI (Moor Instruments, Devon, UK) has a helium-neon gas laser with a 633 nm wavelength of laser light. The coherent laser beam illuminates the field of interest and penetrates through the skin. The laser light hits mobile red blood cells and induces a Doppler shift. The amount of the shift estimates the blood flux and is quantified as an arbitrary unit. The measurable intensity and flux ranges are 0–5000 AU and 0–5000 PU, respectively. The maximum image resolution is 256 × 256 pixels, and the scan speed is around 4 ms/pixel. The scan area varies between 9 × 9 cm and 50 × 50 cm depending on the distance between the detector and the skin. The diameter and divergence of the laser beam are 1.2 mm and 1.4 milliradians, respectively.

2.5 Data analysis with software

The output of Perimed LSCI and Moor Instruments LDPI was analyzed with PIMsoft 1.5 (Perimed AB), MoorLDI2-BI Burn’s Software Version 4.0, respectively.

2.6 Statistical analysis

Data were visualized by generating scatterplots of LDPI and LSCI blood flux against measurement occasion and for each volunteer. Descriptive statistics (mean, standard deviation, and coefficient of variation) were obtained for baseline, biologic zero, and post-iontophoresis maximum blood flux measurements for each device. The biological zero was obtained from the measurement of the stepwise vascular occlusion protocol with 170 mm Hg. Post-iontophoresis maximum blood flux measurement was considered the percentage increase after the baseline value after initiation of iontophoresis. Differences between machines were tested by the Wilcoxon rank-sum test. To model the relationship between LSCI and LDPI, a linear mixed model approach was chosen to account for the clustered nature (repeated measurements for each volunteer) of the data. Linear splines were included to model the non-linear relationship observed in the lower regions of blood flux values. The linear mixed model included random intercept and slopes for the linear splines for each subject assuming an unstructured covariance structure for the random effects. The linear mixed model was optimized by restricted maximum likelihood using the lme4 package (version 1.1-23) in R. Potential bias due to the impossibility to perform the two measurements at exactly the same moment was accounted for by design by alternating the machine that was used first for each subject. To account for the small imbalance due to the difference in group size (n = 8 vs. n = 7), (small) sample weights were included in the regression model to balance groups. The optimal placement of the two knots for the linear splines was examined by considering all possible combinations of placements on a two-by-two grid ranging from 0 to 150 AU with increments of 10. The placements for which the model yielded the smallest Akaike Information Criterion were selected. After fitting the model, it was visually assessed by generating prediction lines for each individual patient based on the best linear unbiased predictions of the random effects (BLUPs). To estimate the correlation between LSCI and LDPI, Pearson correlations were calculated at each measurement occasion and plotted against the mean of the LSCI or LDPI values on each occasion. All analyses were performed using R (version 3.6.3). For all analyses, a two-sided p-value < .05 was used to conclude statistical significance.

3 RESULTS

The median age of the 15 volunteers was 29 years (IQR, 22–40), n = 8 (53%) being female. Vital signs measured just before the study process were as follows: systolic blood pressure 122 ± 10 mm Hg, diastolic blood pressure 75 ± 8 mm Hg, heart rate 77 beats/min, temperature 36.5 ± 0.4°C.

3.1 Baseline, maximum, and minimum blood flux within-subject variability

Baseline blood flux was estimated as 60 ± 11.5 AU at LDPI and 33 ± 6.5 AU at LSCI (p < .001). Moreover, when the biologic zero was extracted from the baseline, the values were still different between the instruments (p = .036). The biologic zero was found to be 29 ± 3.1 AU at LDPI and 10 ± 4.4 AU at LSCI (p < .001). At the end of the iontophoresis protocol, the blood flux reached 724 ± 412% and 260 ± 87% of baseline at LDPI and LSCI, respectively (p < .001). On the other hand, the blood flux decreased by 212 ± 40% and 413 ± 177% from its baseline at LDPI and LSCI, respectively (p < .001). The coefficient of variation at the baseline blood flux was 19% at LDPI and 20% at LSCI.

3.2 Modeling the relationship between LSCI and LDPI blood flux values

The functional relationship between LSCI and LDPI blood flux values was assessed and described by a linear mixed model. The fit of the overall regression line among the observations of all individuals (Figure 2) and individual prediction lines for each individual (Figure 3) are shown. While this relationship can be well described by a single straight line for medium and large blood flux values, a
curvature appears to be present for the lower values (Figures 2 and 3). To take this curvature into account, while keeping the model relatively simple, two linear splines were added to the model. The regression equations describing the relationship are shown (Table 1). Pearson’s correlations between the LDPI and LSCI measurements at each measurement occasion are shown in Figure 4. There was a strong correlation between the LSCI and LDPI instruments at higher blood flux than baseline skin blood flux; however, the correlation decreased at lower blood flux than baseline (Figure 4).

In addition to the linear spline approach, several transformations of the measurements were explored, of which a log transformation of both the LDPI and LSCI measurements appeared to make the relationship almost linear over the entire range of values (Figure S1). This led us to also attempt models regressing the LSCI against the log of the LDPI using a log link function (Figure S2 and Table S2). However, for all models, the linear spline approach (Figure S3 and Table S3) showed better fit statistics and was therefore preferred over the alternative. Results for the linear spline model displayed by gender are shown (Figure S4). Scatterplots showing the iontophoresis and occlusion measurements of LSCI and LDPI at each moment occasion for each volunteer are shown (Figure S5). In addition, Figures with the number of valid measurements at each measurement occasion for each subject have been included and shown at Figure S6.

4 | DISCUSSION

The present study directly compared two commercially available LSCI and LDPI instruments in a healthy population. The main finding is that except for lower blood flux values, the Perimed LSCI and Moor LDPI instruments show a high linear correlation that allows both instruments to be used interchangeably.
FIGURE 3  Scatter plots showing LSCI blood flux measurements against LDPI for all measurement occasions in each volunteer. Solid black dots indicate occlusion, white dots indicate iontophoresis. Individual prediction lines based on the best linear unbiased predictions (BLUPs, dashed lines) obtained from the linear mixed model are shown. The gender of the subject is depicted in the title (f = female, m = male).
The Perimed LSCI device was compared with the Moor LDPI device in healthy volunteers, and a strong linear correlation was shown between different instruments using absolute data except for blood flux lower than baseline values. This finding is in accordance with Millet and colleagues' study, which showed an excellent correlation between the Perimed LSCI and Perimed LDPI. The findings did not differ both in absolute and normalized data in that study. However, they reported this correlation with the same manufacturer's instruments. On the other hand, Stewart et al. investigated the linearity of LSCI and LDPI in the non-healthy population. They reported a high correlation between the Moor LDPI and LSCI in the long term of burn scars, but they also used the same manufacturer's instruments. Each company uses its own AU to define blood flux, and comparing two different companies' devices might be a strength of the research by showing the linearity of data obtained by different algorithms. However, independent of the owner company,

<table>
<thead>
<tr>
<th>Predict AU(LSCI) from AU(LDPI)</th>
<th>Domain (LDPI)</th>
<th>Regression equation for line piece</th>
</tr>
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<tbody>
<tr>
<td>[0,50]</td>
<td></td>
<td>$-12.1 + 0.813 \times \text{AU(LDPI)}$</td>
</tr>
<tr>
<td>[50100]</td>
<td></td>
<td>$10.5 + 0.362 \times \text{AU(LDPI)}$</td>
</tr>
<tr>
<td>[100900]</td>
<td></td>
<td>$33.9 + 0.128 \times \text{AU(LDPI)}$</td>
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<thead>
<tr>
<th>Predict AU(LDPI) from AU(LSCI)</th>
<th>Domain (LSCI)</th>
<th>Regression equation for line piece</th>
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</thead>
<tbody>
<tr>
<td>[0,29]</td>
<td></td>
<td>$14.8 + 1.23 \times \text{AU(LSCI)}$</td>
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<tr>
<td>[29,47]</td>
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<td>$-28.9 + 2.76 \times \text{AU(LSCI)}$</td>
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<tr>
<td>[47149]</td>
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<td>$-264.7 + 7.81 \times \text{AU(LSCI)}$</td>
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Note: Because the regression line contains 2 knots, 3 separate line pieces can be distinguished, for each of which the equations are shown.
comparing LSCI with another laser-based method may not provide equivalent positive results. Tew et al. and Binzioni et al. compared LSCI and laser Doppler flowmetry and reported a poor correlation between these techniques.\textsuperscript{19,20} The underlying reason could be the wide difference in the measured skin surface and the depth of the measured area.

The current study is important for creating a common language between different techniques and manufacturers’ instruments. The underlying reason for comparing different microcirculatory imaging instruments is to underscore the limitations of techniques and find the most suitable way to monitor them. For instance, the Moor LDPI instrument needs several minutes to complete the scanning procedure, which leads to having a low temporal resolution. Although this technique provides a snapshot of microvascular perfusion with a high spatial resolution at a given point, it disables a rapid assessment of the effect of any intervention on blood flow.\textsuperscript{12} Besides, this instrument is quite large in size and has limited mobility. In fact, these issues limit the use of Moor LDPI in clinical practice. As an alternative, the Perimed LSCI could suit for clinical utilization better than LDPI since it allows rapid, high-quality measurement, and maneuverable usage.\textsuperscript{18,21} Using human digits and rabbit muscles, Forrester et al. compared laser speckle contrast techniques with LDPI, and they suggested that laser speckle contrast techniques are an acceptable and fast alternative for LDPI. Also, having a higher temporal resolution enables laser speckle contrast techniques to be more sensitive to assess rapid changes in blood flux.\textsuperscript{22} Indeed, we observed in the current study that the Perimed LSCI was more practical in use; however, was able to measure smaller surfaces than LDPI.

Another main finding of the current study is that the correlation of both instruments was not as strong during lower blood flux values. Once the arm was occluded, the blood flux went down and almost reached similar ranges for all occlusion steps. Losing linearity between both techniques during the stepwise vascular occlusion test might be explained by several mechanisms. Firstly, LSCI and LDPI devices do not have similar sensitivity to detect skin blood flux at low blood flux levels. For example, Tew et al.\textsuperscript{19} demonstrated that the LSCI device is more sensitive to changes in RBC velocity than RBC concentration in the cold pressor test. Similarly, Draijer proved that the relation between speckle velocity and contrast is not linear.\textsuperscript{23} Therefore, the change in RBC velocity might not be parallel with the change in RBC concentration, and a non-linear association could also be detected. Secondly, skin blood flux values almost decreased to half of the baseline at occlusion with 50 mm Hg and did not show a major change during the occlusion at 80, 110, 140, and 170 mm Hg. Therefore, the correlation analysis was performed within a narrow range of blood flux, where the range gets even narrower if the Brownian motion is extracted from the flux level. This finding is interesting since having a low blood flux even with 50 mm Hg occlusion bears in mind that the veno-arterial reflex might also contribute to lowering the blood flux regardless of the occlusion.\textsuperscript{24} This is an important finding and should be considered in clinics, especially in patients with congestion.

Having a strong correlation is crucial for using the instruments interchangeably, especially in specific clinical situations. For instance, Moor LDPI is the only validated monitoring technique for evaluating burn depth to better decide treatment modality in burn patients. Niazi et al.\textsuperscript{25} reported that the burn depth assessed by biopsy specimen has excellent agreement with LDPI, where the agreement was only 65% with clinical criteria alone. Thereafter, an LDPI color code was created and used in the clinic to help burn surgeons decide whether they need surgical or conservative management.\textsuperscript{26} An accurate interpretation of the LDPI color code indicates wound healing potential.\textsuperscript{27,28} Considering the already mentioned advantages of the LSCI technique, the current study might be a pilot for clinical research to create a similar color code for the LSCI to be a basis for burn patients’ management.

From the technical point of view, both Perimed LSCI and Moor LDPI instruments display a similar principle to indicate blood flux levels. The dark blue and red correspond to the lowest and the highest blood flux, respectively. The colors between dark blue and red correspond to the values in between. The Perimed LSCI instrument has a 256-level color scale; however, Moor LDPI has a 6-level color scale. In this study, a regression equation was created, which could help clinicians easily interpret the blood flux findings. Bear in mind that the color code of each technique does not display exactly equivalent blood flux values. The underlying reason for this issue is that the measured depth depends on the optical properties of the tissue and laser light’s wavelength, which is 633 nm in Moor LDPI and 785 nm in Perimed LSCI.\textsuperscript{12} So, the measured depth is assumed as ~1-1.5 mm with Moor LDPI and ~0.3 mm with Perimed LSCI.\textsuperscript{12,21} Further research is needed to create a relevant color code to be used in clinical practice.

The current study implemented the sodium nitroprusside-iontophoresis technique as the provocation test. Sodium nitroprusside is a nitric oxide donor leading to vasodilatation in an endothelium-independent way.\textsuperscript{29} It increases microvascular blood flux by inducing smooth muscle relaxation through an increase in cyclic guanosine monophosphate synthesis.\textsuperscript{29} There were differences among the volunteers in their response to the sodium nitroprusside. Ethnicity, gender (even use of oral contraceptives in women), age, and microvascular reactivity capability might be responsible for observing a different range of increases in skin blood flux. There are various provocation tests to assess different compartments of the microvascular system. For instance, acetylcholine-iontophoresis is used to test endothelium-dependent microvascular response, and post-occlusive reactive hyperemia test is used to study the involvement of sensory nerves.\textsuperscript{10,31,32} Microvascular blood flux capacity, also known as microvascular reactivity, precedes cardiovascular disease like atherosclerosis.\textsuperscript{33} In line with the current study, Humeau-Heurtier et al. suggested that generating algorithms and concurrently using different techniques might enable diagnosing and predicting microvascular and cardiovascular disease and understanding the physiopathological process between macro- and microvascular systems.\textsuperscript{7}
4.1 | Limitations

The current study has limitations. Firstly, a simultaneous assessment of the skin blood flux could not be performed, fearing that laser lights of different LBTs could have interference. Indeed, the implicit assumption is made that the two measurements were conducted simultaneously. In reality, the measurements were conducted sequentially. To avoid directional bias, the order of the devices was alternated between volunteers, and a small regression weight was added to account for the odd number of volunteers. Despite these precautions, the non-simultaneous measuring will still add to the measurement error. Accordingly, we could not perform a Bland-Altman analysis to test agreement between the instruments, since we could not simultaneously record the blood flux and both techniques use different AUs. Secondly, LSCI and LDPI measurements are affected by many factors such as temperature, air movement, scanning distance, laser wavelength, tissue optical characteristics, and skin color.34 However, all these environmental, volunteer, and instrument-related factors were strictly controlled in the current study. Due to the skin temperature impacting blood flux and vasodilatory response to the sodium nitroprusside iontophoresis test, all volunteers underwent the protocols in the same room with a constant temperature.35 In this study, a heterogeneous population with different ethnicities was enrolled so that the results could be generalized.

Lastly, despite the high correlation, especially in the higher range of blood flux values one may perceive the 95%-prediction interval as rather wide. This could introduce substantial error at the individual level when making translations from one machine to the other by a regression equation based on the population mean trend. However, whether the error is substantial enough to affect clinical decision-making cannot be answered from this data. For this, the impact of color translation on images of burn wounds obtained by both machines should be assessed. Ideally by comparing whether images of the same wound by different machines would lead to different clinical decisions. Especially in the higher ranges, a deviation may not be consequential since it would still lead to the same conclusion that blood flow is adequate.

5 | CONCLUSION

Perimed Pericam LSCI (Perimed AB) is highly comparable to the Moor LDPI instrument (MoorLDI2-Bi, Moor Instruments) except for blood flux lower than baseline values. This study’s findings could be a basis for the interchangeable use of both instruments in healthy populations.

6 | PERSPECTIVES

In the future, it would be valuable to test the comparability of these devices in specific patient populations, notably in burn patients. So, further research is needed to integrate these techniques into clinical practice and decision-making.

AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST
None.

DATA AVAILABILITY STATEMENT
The authors will make the data available upon request to the authors (drgoksel@hotmail.com).

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REFERENCES


SUPPORTING INFORMATION
Additional supporting information can be found online in the Supporting Information section at the end of this article.

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