# Validation of a Non-invasive Imaging Photoplethysmography Device to Assess Plantar Skin Perfusion in Comparison with Laser Speckle Contrast Analysis.

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# Validation of a Non-invasive Imaging Photoplethysmography Device to Assess Plantar Skin Perfusion in Comparison with Laser Speckle Contrast Analysis.

Assessing skin perfusion is an established and reliable method to study impaired lower limb blood flow. Laser Speckle Contrast Analysis (LASCA) has been identified as the current gold standard to measure skin perfusion. Imaging photoplethysmography (iPPG) is a new low-cost imaging technique to assess perfusion. However, it is unclear how results obtained from this technique compare against that of LASCA at plantar skin. Therefore, the aim was to investigate the association between the skin perfusion at the plantar surface of the foot using iPPG and LASCA. Perfusion at six plantar locations (Hallux, 1st 3rd 5th metatarsal heads, midfoot, heel) was simultaneously measures using LASCA and iPPG in 20 healthy participants. Skin thickness and skin temperature were also collected at the same plantar locations. Spearman's rank tests showed significant associations with medium strength between the perfusion values measured with LASCA and iPPG for most tested sites. No improvement in the relationship between iPPG and LASCA data was observed when controlling for either skin thickness or skin temperature. Skin perfusion values obtained using iPPG were found to be significantly associated with the corresponding values obtained using the gold standard LASCA device. Additionally, the measurement of perfusion using iPPG is shown to be robust.

Imaging Photoplethysmography, Laser Speckle, Microvascular, Skin Perfusion, Plantar soft tissue

## 1. Introduction

Assessing skin perfusion is an established and reliable method to study impaired lower limb blood flow [1–3]. Perfusion is a measurement of the rate at which blood is delivered to the tissue [4]. However, measurements of perfusion are a particularly challenging physiological measurement due to the spatial inhomogeneity of vessel structure and the variability of perfusion over time [5].

Common techniques used in the measurement of perfusion include laser Doppler, Laser Speckle Contrast Analysis (LASCA), and nailfold capillaroscopy [5]. Additionally, there are several emerging technologies aimed at measuring perfusion. These include imaging photoplethysmography, optical coherence tomography, photoacoustic tomography, hyperspectral imaging, and tissue viability imaging [5].

Recent literature regarding the measurement of perfusion has focused on the use of perfusion measurement devices, particularly LASCA [6,7]. Laser Speckle Contrast Analysis allows for a single-shot, full-field image of microvascular perfusion and works by exploiting the interference pattern generated by the backscattering of diffuse light from the skin [8]. Laser speckle contrast techniques are based on the spatial and temporal statistics of the speckle pattern. When the skin is illuminated using coherent laser light, the laser light is reflected by the skin's red blood cells. The reflected light creates light and dark areas, which create what is known as a speckle pattern [9].

In perfused skin, the red blood cells' movement causes changes in the speckle pattern generated and forms a dynamic speckle pattern. The dynamic character of the speckles can be visualised with a camera with an integration time longer than the time scale of the speckle fluctuations, causing the speckle pattern to be blurred on the recorded image [9–11]. Increased perfusion (i.e. increased local concentration and/or increased speed of moving red blood cells) result in a reduced spatial speckle contrast [9,12]. Fluctuations in the speckle pattern and the reduction in contrast of the speckle pattern is, to an extent, related to the speed of the illuminated objects, in this case, the red blood cells within the skin [9].

While LASCA has been widely validated against other established methods of measuring microvascular perfusion [13–17], one of its main limitations is the cost of the device which limits the availability of the technique to clinicians. Therefore, a low-cost alternative to measuring microvascular perfusion could aid in increasing the use of perfusion measurements.

An alternative to LASCA is the use of Imaging Photoplethysmography (PPG). Imaging PPG (iPPG) is an emerging, non-contact method that can detect heart-generated pulse waves utilising peripheral blood perfusion measurements. It can capture spatial information from multiple sites simultaneously [5,18–21]. In its most basic form, Imaging Photoplethysmography requires only two components: a light source to illuminate the tissue and a photodetector to measure small variations in light intensity. The light interaction with the illuminated tissue is complex with variables such as light transmission, reflection, absorption, multiple scattering, and fluorescence all affecting the interaction [22,23]. Additionally, blood volume, blood vessel wall movement, and the orientation of red blood cells can affect the returning light intensity received by the photodetector [24–26]

Imaging Photoplethysmography measurement systems usually operate in what is known as reflection mode [5]. In reflection mode, both the illuminating light source and photodetector are situated alongside each other [5,18–21]. In reflection mode, the interaction between the light and the tissue being imaged depends on the penetration depth of the illuminating light [19–21]. Red and infrared light have been shown to penetrate the tissue for several millimetres, e.g., 2.5mm at the wavelength of 810nm [27] while other light sources such as green light will only penetrate the tissue up to 1mm

[23,27]. Based on this, when using a green light source, the measurement obtained iPPG can be said to be a measurement of skin microvascular perfusion [21,23,27].

However, assuming that the measurement of iPPG using green light is a measurement of skin perfusion, the skin's properties, such as skin hardness, skin thickness, and skin temperature, may affect the measurement of perfusion using iPPG and must be taken into account. Current literature suggests that as the skin temperature decreases the penetration of light into the skin increases indicating cooling-induced transparency of skin [28]. Therefore, any differences in the measured temperature of the foot need to be accounted for as differences in the temperature of the skin could affect the measurement of perfusion by measuring different structures of the plantar soft tissue.

Additionally, the effect of skin hardness must be assessed. It is suggested that collagen cross-linking causes an increase in the hardness of the skin on the plantar surface of the foot [29,30]. This cross-linking of the collagen fibres could increase the skin's density and thus decrease the penetration of the green light into the underlying skin where the blood vessels are located. By assessing the effect of skin hardness on the measurement of perfusion, the effect of potential collagen cross-linking can be taken into account. Finally, as highlighted previously, the penetration of the green light used in iPPG is less than that of the LASCA. Hence assessing skin thickness in addition to skin hardness ensures that the measurement of iPPG falls within the skin thickness limit.

Therefore, this study aimed to investigate if the plantar surface skin perfusion measured using a low-cost green light iPPG system is associated with that of the gold standard LASCA.

## 2. Methodology

For this study, an iPPG perfusion device was developed in association with Cadscan (Cadscan, Chester, United Kingdom). This prototype perfusion camera is designed to be used in reflectance mode. An Intel® RealSense Depth Camera (Intel®, California, United States) was used as the photodetector [Figure 1]. The plantar surface of the foot was illuminated using a green LED of wavelength 540nm was used [31]. The frame rate of video recording was 30 frames per second (fps), and the frame size was 1280 × 720 pixels. Recording time was set to be 300 frames (10 seconds) with the duration of recording selected based on previous work by Kamshilin et al [20]. This video duration of 300 frames (10 seconds) corresponds approximately to 10 periods of the cardiac cycle and was found based to be able to represent the most important spatial distribution features with regards to the perfusion of blood within the foot [20]. The intensity of ambient illumination in the laboratory was much lower than that provided by the LED illuminator.

After necessary approval from the ethics review committee at Staffordshire University, 20 healthy participants with no history of recent major lower limb surgery or neurological disorders were recruited from the local population. This study's sample size was determined based on sample size calculations provided by G*Power (version 3.1.9.6) [32,33]. As this study investigates the association between LASCA and iPPG correlation testing was selected. A large effect size of $\rho=0.6$ was selected with $\alpha=0.05$ and power=0.8. The correlation sample size calculation based on these parameters indicated the 17 participants as the minimum required number of participants .

All participants provided full written informed consent before commencing testing. Demographical data were recorded through a patient-led questionnaire, including questions related to their general health, footwear, and history of foot-related pathologies.

To validate the prototype iPPG perfusion camera, the participant was asked to lie supine on a medical couch in a temperature-controlled room (21.5°C±1.5°C) for 10 minutes before testing. This acclimatisation period of 10 minutes has been established to be adequate to allow the skin temperature to reach a constant value [34,35]. After the acclimatisation period, the participant was asked, while remaining in a supine position to place their feet at the end of the examination couch [Figure 1]. The prototype perfusion (Photoplethysmography PPG) camera was then placed approximately between 40-45cm away from the foot, dependent on the foot size, to enable the entire length of the foot to be captured. This distance from the foot to the perfusion camera was measured, and the head of the Laser Speckle Contrast Imager (Pericam, Perimed AB, Jarfalla, Sweden) was placed the same distance from the foot as that of the perfusion camera [Figure 1].

As both the LASCA and perfusion camera work based on reflected light, care was taken to ensure that both cameras were positioned as square to the foot as possible ( with thee cameras optical axes perpendicular to the plantar surface) to minimise parallax errors. Ideally both LASCA and Perfusion camera should have the same position in relation to the plantar aspect of the foot where the measurements are taken place. While given the requirement for simultaneous measurements, this was not feasible, it was made sure that the LASCA and Perfusion camera are aligned with respect to the longitudinal axis of the foot to minimise such error [Figure 1]

Correct alignment of the cameras to the foot was crucial as misalignment lead to the measured levels of perfusion being lower due to less light being reflected back to the photodetectors, which therefore could have affected the calculation of perfusion.

A series of three, 30 second, recordings were taken, for the left foot [Figure 2]. To ensure consistent measurements, a sheet of black foam was placed over the ankle of the participant. This provided a consistent baseline condition for both the LASCA and

the perfusion camera, helping to reduce the noise within the images captured and also ensured that only the foot was being recorded [Figure 1].

In addition to the measurements of perfusion, parameters related to temperature and skin properties were collected. Thermal images were taken to assess the skin temperature at the plantar aspect of the foot. This camera was positioned in such a way to be able to capture the whole length of the foot [Figure 1].

The measurement of skin hardness was conducted using a Shore-00 hardness device (Shore 00, AD-100, Checkline Europe B.V, Dennenweg, The Netherlands). The participant was asked to lie in the prone position face down on the medical couch with their shank (shin) in the air approximately 90 degrees to the thigh. The durometer was lowered with the foot relaxed onto the following plantar sites: Hallux, 1$^{st}$ metatarsal head, 3$^{rd}$ metatarsal head, 5$^{th}$ metatarsal head, midfoot, and heel. The tissue under the durometer was allowed to be compressed by the full weight of the device before taking the reading of hardness. Each site is tested three times, and an average value of Shore hardness for each site calculated.

Ultrasound imaging was utilised to assess the skin thickness at the following plantar sites: Hallux, 1$^{st}$ metatarsal head, 3$^{rd}$ metatarsal head, 5$^{th}$ metatarsal head, midfoot, and heel. To conduct the test, a large gel pad, formed of ultrasound conductive gel, was placed between the probe and the skin. This was to aid in minimising the compression of soft tissues surrounding the plantar sites. A series of static images were captured for analysis whereby the thickness of the skin was determined. Measurements were taken at 3 locations located at the left, centre, and right of the ultrasound image used to collect skin thickness.

### *2.1. Data analysis*

To aid in the comparison of the data obtained by the LASCA and the perfusion imaging camera, a bespoke data analysis tool has been developed at Staffordshire University. This data analysis tool is based on a data extraction tool provided by Perimed, the manufacture of the LASCA. This data analysis tool allows for the manipulation of the obtained images to ensure that both images are the same pixel size and in the same orientation. The analysis tool also allows for a direct comparison between the LASCA data and that recorded using the perfusion device by devising a correlation matrix whereby the agreement between the two images can be assessed. This method allows for a direct comparison between images collected using the LASCA and the iPPG camera in the areas of the Hallux, 1$^{st}$ Metatarsal head, 3$^{rd}$ Metatarsal head, 5$^{th}$ Metatarsal head, lateral midfoot, and the heel.

Shapiro-Wilk test was used to screen the data for normal distribution ($p<0.05$). The parameters of skin thickness, Shore hardness, and thermography were found to be normally distributed. However, the measurement of skin perfusion measured using the perfusion camera and LASCA were found to be non-normally distributed at all anatomical sites of interest (Hallux, 1st Metatarsal head, 3rd Metatarsal head, 5th Metatarsal head, lateral midfoot, and the heel). Therefore, non-parametric tests were employed. All statistical analyses were conducted using commercially available software (IBM® SPSS®v.25). Partial correlation tests were used to account for the parameters related to skin and temperature.

## 3. Results

The 20 participants (9/11, M/F) recruited for this study had an average age of 38(±10) years. The highest variability and average measurement of perfusion using the LASCA

were found at the hallux [Table 1] with the lowest perfusion value found at the midfoot. For the measurement of perfusion using the iPPG system, the highest average value of perfusion was found at the 1st metatarsal head with the highest variability at the 3rd metatarsal head. The lowest average value of perfusion using the iPPG system was found at the heel.

Significant correlations with medium strength (except for the 1st Metatarsal head) were observed between the perfusion measured using LASCA against those obtained from iPPG [Table 2]. When partial correlation which took into account the effect of skin thickness, harness and temperature were used, no improvement in the relationship between PPG and LASCA data was observed.

## 4. Discussion

Based on current literature regarding the measurement of microvascular perfusion, there have currently been no studies which have looked to compare or validate imaging photoplethysmography against that of the gold standard Laser Speckle Contrast Analysis (LASCA). This study shows for the first time that there is a clear significant association between the values obtained by imaging photoplethysmography (iPPG) compared to the gold standard LASCA device. Except for the 1st metatarsal head, all other sites across the plantar surface of the foot showed medium-strength correlations with the results of the LASCA.

To further understand the association between LASCA and iPPG the physical meaning of each measurement obtained by LASCA and iPPG [Table 2] must be explored. For LASCA, the measurement obtained is expressed as perfusion units. Perfusion units are an arbitrary measurement that, as previously mentioned, is based on the blurring of

the spatial speckle pattern [9–11] and the change in spatial speckle contrast [9,12]. Whereby a higher value of perfusion unit is, to an extent, related to an increase in perfusion. Currently, within the literature there no theoretical model that can directly link changes in the spatial speckle contrast to changes in perfusion. However, there is generally agreed on assumption within the literature that a decrease in spatial speckle contrast is associated with increased red blood cell velocity and, therefore, perfusion [9,24,36,37].

For the value of perfusion obtained by the iPPG device, this is a dimensionless value that is related to red blood cell-volume pulsations within a tissue at the mean heart rate. The higher the amplitude of the red blood cell pulsations recorded, the higher the volume of red blood cells provided to the tissue and, therefore, a larger value of perfusion.

Whilst LASCA and iPPG can be said to be measuring two separate measurements namely the red blood cell velocity (LASCA) and red blood cell volume (iPPG) they can both be said to be measuring perfusion. Perfusion defined is the rate at which blood is supplied to the soft tissues of the body.

Due to the difference in the wavelength of infrared light used in LASCA versus that of the green light that is used in iPPG, the penetration depth of LASCA would be deeper compared to that of the iPPG. Hence compared to the LASCA device, the iPPG measures perfusion at a shallower skin depth.

However, when the effect of skin thickness, skin stiffness, and temperature was considered, no improvement in the relationship between iPPG and LASCA was observed. This can indicate that the association between the measurements are not affected by these confounding variables.

When looking at the properties of the skin, in particular, the thickness of the skin across the plantar surface of the foot the average thickness was found to be 0.07±0.01cm and is in line with previous research, investigating skin thickness in healthy participants [38]. As previously mentioned the average penetration depth of green light into the skin is approximately 1mm (0.10 cm) [23,27] while for the infrared light used by the LASCA, the penetration depth into the skin is approximately 2.5mm. In comparison, the measurements of perfusion using the LASCA will be a measurement of perfusion in the dermal layer of the plantar soft tissues due to its larger penetration depth [27,38]. Therefore, the measurement of perfusion using the iPPG system is a more representative measurement of plantar skin microvascular perfusion compared to that of LASCA.

The ability to say that the measurement of perfusion using the iPPG system is a measurement of skin perfusion and that the measurement is linked to that of LASCA allows for this approach to be used in various situations. This may aid in the assessment of wound healing. Laser speckle contrast analysis has been an important tool in the study of wound healing. In particular, in the case of burns, it was found that burns that healed within 14 days were found to have higher perfusion compared to burns that took more than 14 days to heal or ultimately required surgery [39]. Additionally, this prototype camera may find uses in the treatment and care of diabetic foot ulcers. The current research has shown that ulcers that have good perfusion around the wound are more likely to heal and thus prevent amputation [6,40–42]. Currently, there are no low cost clinically viable methods to measure the perfusion of the foot; thus, this prototype perfusion device may bridge that gap and aid in the treatment and prevention of foot ulcers.

However, one of the main limitations of this study is that it was limited to healthy participants though this is a necessary first step to initially validate the concept. The findings of this study need to be validated in a clinical population, such as those with

diabetes or burns, to ensure that this link between LASCA and iPPG is consistent between groups. Due to the non-invasive nature of this testing protocol, this will allow for large scale cohort studies to be performed with minimal impact on participants.

In conclusion, based on the results of this validation study, there is a significant association between the values obtained by this new perfusion imaging (PPG) camera when compared to the gold standard LASCA device. Additionally, due to the penetration depth of green light, it can be said that the perfusion measured using this new iPPG camera is that of skin microvascular perfusion. Future research is however required to investigate if this link between LASCA and iPPG exists in clinical populations such as those with diabetes and how the device can be best implemented clinically.

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Declaration of Interest

The authors report no conflict of interest

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Table 1: The average value and standard deviation of thermography, Shore hardness, and skin thickness for each of the plantar sites tested

| | ***Thermography (°C)*** | ***Shore-00 hardness*** | ***Skin thickness (cm)*** |
|---|---|---|---|
| *Hallux* | 28.39 ± 4.2 | 23 ± 8 | 0.07 ± 0.02 |
| *1st Metatarsal Head* | 29.79 ± 3.14 | 28 ± 12 | 0.07 ± 0.01 |
| *3rd Metatarsal Head* | 29.68 ± 2.75 | 19 ± 10 | 0.07 ± 0.01 |
| *5th Metatarsal Head* | 29.25 ± 2.8 | 27 ± 10 | 0.07 ± 0.01 |
| *Midfoot* | 29.95 ± 2.23 | 23 ± 6 | 0.06 ± 0.01 |
| *Heel* | 29.65 ± 2.68 | 34 ± 7 | 0.06 ± 0.01 |

Table 2: Laser Speckle (LASCA) and Plethysmography (PPG) average values and standard deviation. In addition the results of correlation testing are presented.

| ***Left Foot*** | ***LASCA*** | ***PPG*** | ***Correlation*** |
|---|---|---|---|
| *Hallux* | 69.03±46.61 | 2.53±1.61 | r=0.635, p=0.003 |
| *1st Metatarsal Head* | 59.86±31.36 | 3.29±2.37 | r=0.38, p0.098 |
| *3rd Metatarsal Head* | 55.32±27.72 | 3.06±2.87 | r=0.58, p=0.007 |
| *5th Metatarsal Head* | 57.48±30.33 | 2.22±1.75 | r=0.612, p=0.004 |
| *Midfoot* | 42.71±21.77 | 2.05±1.45 | r=0.683, p=0.001 |
| *Heel* | 53.97±32.9 | 1.76±1.74 | r=0.646, p=0.002 |
| *Whole foot* | 56.4±30.16 | 2.49±1.79 | r=0.648, p=0.002 |

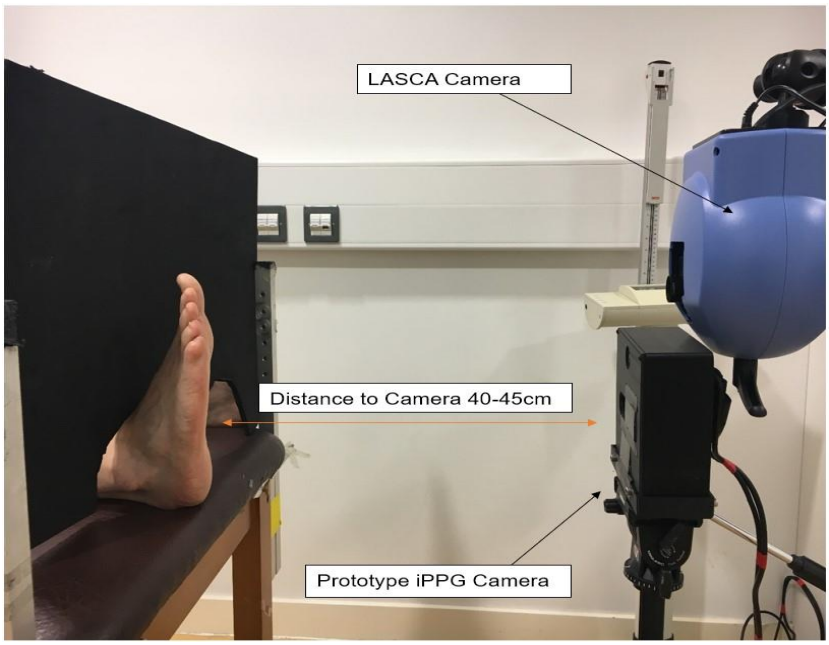


Figure 1. Testing setup for the collection of perfusion data using the Laser Speckle Contrast Imager and the prototype iPPG camera system.

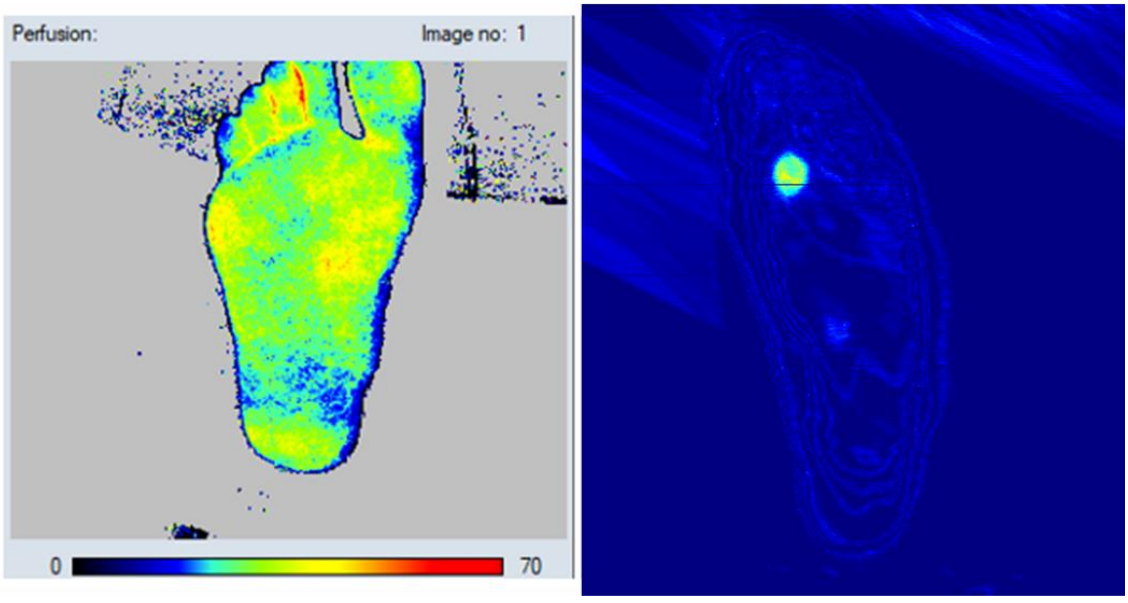


Figure 2. A) Example image of LASCA output after recording B) Example image of iPPG perfusion after data capture.