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Effect of skin optical absorption on speckleplethysmographic (SPG) signals

TYLER B. RICE,* BRUCE YANG, AND SEAN WHITE

Laser Associated Sciences, Inc., CA 92612, USA
tylerrice@laserassociatedsciences.com

Abstract: Recent advances in optical technology have emerged for measuring blood flow in the extremities using speckleplethysmography (SPG), which may address needs in vascular medicine and other fields. SPG has demonstrated a highly linear response with flow rate, but the susceptibility to differences in skin tone is unclear. Two validation studies using skin-simulating phantoms and a simple clinical protocol were conducted to determine the impact of absorbing skin layers on SPG measurements. Benchtop results demonstrated that the coefficient of determination between known flow rate and SPG was highly linear (R^2 = 0.990) and was unaffected by the addition of skin-phantom layers with variable absorption (R^2 = 0.996-0.999). Additionally, no significant trend was found between the fit residuals of SPG and flow rate with increasing skin-phantom absorption (R^2=0.025, p = 0.29). In clinical testing, no significant difference was found using both a 4-way ANOVA between demographic classifications (F = 0.89, p = 0.45), and a 2-way ANOVA test between lower- and higher-melanin subclassifications (F = 0.4, p = 0.52).

1. Introduction

The nature of blood flow in the extremities is highly dynamic and a key metric of systemic tissue health. Abnormalities in the peripheral flow often manifest as the first response to more serious vascular impairment [1]. For example, Critical Limb Ischemia (CLI), caused by Peripheral Artery Disease (PAD), is the highest contributor to lower limb amputation, representing significant cost burden to the US healthcare system [2]. Improved diagnosis and daily monitoring has been shown to reduce the number of amputations by over 50% [3]. Blood flow to the extremities is also relevant in dialysis [4], sleep apnea [5], endothelial dysfunction [6], and sports medicine [7].

Despite its importance, current technologies used to measure peripheral blood flow have significant drawbacks that hinder adoption. One common optical technique, Laser Doppler Flowmetry (LDF), directly monitors the movement of red blood cells in the microvasculature using dynamic light scattering [8]. However, LDF is a reflectance measurement and thus restricted to monitoring superficial cutaneous blood flow [9]. LDF lacks repeatability caused by spatial inhomogeneity in the flow signal and skin thickness [10,11]. Photoplethysmography (PPG) is a measurement of hemoglobin absorption but is often claimed to be a proxy to blood flow. However, PPG relies on the elastic expansion and contraction of vessels during the cardiac cycle and is not a direct measurement of flow. During periods of significant vasoconstriction and low perfusion, PPG amplitude becomes significantly reduced or may entirely disappear [12].

An increasingly common technique, Laser Speckle Imaging (LSI), has recently been used in a transmission geometry to record blood flow in the extremities. LSI utilizes a laser source and image sensor to record the interference pattern, referred to as speckle, generated by scattered coherent light. The transmission geometry provides highly diffuse signals that probe the full tissue thickness, and flow is determined using metrics of pixel contrast to gauge the speckle fluctuation rate [13,14]. Ghijsen et al. demonstrated that transmission LSI was highly linear (R^2 = 0.98) with flow rate in a benchtop validation test under controlled flow conditions [15]. When tested in vivo, the technique produced a high-resolution waveform of flow during the cardiac cycle, referred to as the Speckleplethysmogram (SPG). In a study of volunteers undergoing a cold

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pressor challenge, the SPG waveform maintained a robust signal-to-noise ratio (SNR) under conditions of significant vasoconstriction relative to PPG, which failed to provide any reading [15,16]. SPG has also shown improved accuracy in determining heart rate variability over PPG [17].

SPG analysis is a promising new diagnostic tool to assess both the adequacy and dynamics of blood flow in the extremities. However, little is known about the impact of turbid skin layers on the accuracy of measured flow values. In the visible and near infrared light spectrum, melanin is a significant optical absorber in skin. Melanin varies in concentration among demographic groups, which may affect the accuracy of some spectroscopic techniques due to the resulting variation in light absorption [18]. The impact of melanin concentration on SPG is important to assess for continued clinical adoption of the technology.

Generally, reflectance-based LSI technologies are sensitive to absorbing surface layers with static scattering components such as skin [13]. Researchers have previously attempted to compensate for static layers through Monte Carlo modeling techniques [19]. In contrast, transmissive LSI may be naturally resistant to these effects since detected photons have necessarily traveled through a dynamic scattering layer [20,21]. Herein we examine the empirical effect (or lack thereof) of turbid skin layers with varying absorption on measured flow readings. Use of SPG as a clinical diagnostic tool is also discussed. Results are collected from both a benchtop validation model using skin simulating phantoms and a clinical feasibility study.

2. Materials and methods

2.1. SPG instrument

The instrument used to generate the SPG waveform was a commercially available device based on Affixed Transmission Speckle Analysis (ATSA) technology (FlowMet-R, Medtronic PLC, Minneapolis, MN). ATSA is an embodiment of LSI that allows for robust generation of SPG waveforms because the source and detector are integrated into the housing and affixed directly to the sample. This improves consistency of placement and reduces motion artifact when compared to a non-contact imager [15]. An illustration of the instrument clipped onto a toe is shown in Fig. 1. Within the housing are a CMOS sensor array and a 785 nm laser diode placed opposite one another in a transmission geometry. Images were recorded at approximately 240 Hz. Speckle contrast was calculated and converted to flow values using techniques ubiquitous in LSI [13,15]. The resulting flow waveform, SPG, was then saved for analysis.

2.2. Benchtop test setup

The model for testing the effect of skin on flow readings described herein was developed based upon the validation setup implemented by Ghijsen et al. [15], where the linearity of transmission LSI measurements were compared against known volumetric flow rates in the physiological range. First, a finger analog was synthesized using silicone rubber tubing encased in skin-simulating layers of varying absorption values, as illustrated in Fig. 2. A blood-simulating fluid phantom was then created that could be pumped through the tube at specified flow rates. The fluid and skin-simulating phantom media optical properties were chosen to mimic the averages of bulk tissue at 785 nm [22,23], and set using specific concentrations of absorbing nigrosine dye and scattering intralipid particulates described in the biomedical optics literature [24,25]. Specifically, the fluid scattering and absorption properties were set at $\mu_s' = 1.0\,mm^{-1}$ and $\mu_a' = 0.1\,mm^{-1}$ using a dilution of 1% intralipid and 0.005% nigrosine dye, respectively. The skin-simulating phantom layers were created with a base of silicone rubber cast to 1.1 mm thick, approximating epidermal-dermal thickness of human digits (0.8–1.44 mm) [26]. The scattering coefficients of the skin phantom layers were set mixing titanium dioxide to $\mu_s' = 1.0\,mm^{-1}$ [24]. The absorption coefficients of the skin phantom layers were varied using differing concentrations of powdered nigrosin dye. To

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Fig. 1. Illustration of the SPG instrument clipped onto a subject's great toe. Coherent light from the laser diode scatters within the tissue (indicated by the red arrow) and is recorded by an image sensor on the opposing side.

ensure the full range of melanin values were encompassed in the test, the absorption coefficients of the analogs were varied empirically to achieve target reductions in transmitted light intensity. Mean transmitted intensity was measured through five different analogs chosen to encompass the dynamic range of the FlowMet specification, from 20 to 200 counts, equating to a normalized absorption range of 0-90%. This absorption range is set to fully encompass the possible range due to melanin absorption, which has been estimated to be 0-31%, depending on epidermal thickness [27]. A tubing diameter of 19 mm was chosen to approximate the average diameter of a human digit [28], and connected in series to a syringe pump (Pump Systems, Inc.). The blood-simulating fluid medium was pumped through the tube at flow rates approximating the physiological range of a human digit (2–20 ml/min), in steps of 2 ml/min [29]. For each skin phantom layer, flow was varied every 10 seconds in intervals of 2 mL/min up to a maximum 20 mL/min using the electronic controls of the syringe pump. The flow response and correlation to absorption values was then determined.

2.3. Clinical test protocol

A cross-sectional pilot study was conducted at an outpatient vascular clinic in Orange, CA. All measurements were performed with IRB approval (Western IRB, Pallyup MA). Any patient visiting for routine noninvasive arterial assessment was eligible for participation. The approved protocol was conducted in a vascular lab where SPG measurements were obtained during routine standard of care. Basic demographic information was collected from participants enrolled in the study who then received vascular testing and an SPG recording.

2.3.1. Demographics

Standard demographic information was collected and used to estimate skin melanin content. Participant classification groups included: White/Caucasian, Asian, Black/African American, and nonwhite Hispanic/Latino. Although the distribution of melanin content can overlap

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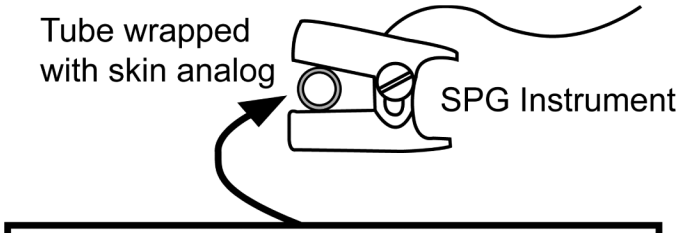

Trial	Skin Analog #	Tube Appearance
1	1	
2	2	
3	3	
4	4	
5	5	
6	None	

Fig. 2. Benchtop validation setup. The SPG instrument is placed on a tube with known flow. Silicone phantom layers of increasing absorption are placed around the tube to simulate varying skin types.

between classifications, mean differences in spectral intensity due to melanin volume fraction has been reported in the literature among the standard demographics [30,31]. To maximize statistical power, a binary classification with wider demographic groupings of lower melanin (White/Caucasion) and higher melanin (Asian, African American, and nonwhite Hispanic/Latino) was also performed.

2.3.2. Vascular tests

Patients were first acclimated for five minutes in a resting state according to standard of care for obtaining lower extremity pressure measurements [32]. Following the rest period, a 30-second SPG measurement was acquired from the great toe using the FlowMet device. This site was chosen based on its direct clinical applicability for patients with peripheral artery disease (PAD) where physicians seek to evaluate blood flow quality in the lower extremity digits. Mean flow data was calculated across the full length of the measurement. Toe pressure measurements were then obtained using standard pressure cuffs and a doppler probe to record pulses.

Because flow is proportional to toe pressure values for a given vascular resistance, toe pressure measurements were used to control for any demographic bias of blood flow values. For example, there may be significant correlations between demographic group and decreased toe pressure and blood flow values due to a higher prevalence of peripheral artery disease (or other natural physiological variability). Significant flow differences between groups that are not reflected in toe pressure are thus possible measurement errors arising from skin tone.

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2.4. Statistical analysis

Quantitative variables are reported as mean and standard deviation (SD). Categorical variables are reported as frequency and percentage. The R^2 value between flow measurements and syringe pump output was determined for each skin analog absorption value through a simple linear regression and compared. The correlation coefficient between skin analog absorption values and measured flow was also compared. Flow and toe pressure measurements taken from the clinical protocol were classified according to demographic breakdown as described above. Analysis of Variance (ANOVA) tests were performed for mean flow and toe pressure measurements within and between each ethnic group. An additional ANOVA test was conducted between the lowest melanin (white) and higher melanin (nonwhite) groups. Toe pressures were used to control for potential demographic bias of PAD prevalence.

3. Results

3.1. Benchtop validation

Mean intensity absorption for each of the five skin phantoms tested showed normalized reductions of 41%, 55%, 73%, 78% and 90%. Flow response curves as measured by the SPG device for each of the skin analogs and the no-analog (0%) case are shown alongside the linear best fit of the aggregate data in Fig. 3(a). The fit was found to be highly linear (R^2=0.99, $p<0.01$), in line with the specification provided by the manufacturer. Individual response curves for the five skin phantom cases showed no significant reduction in linearity (R^2=0.996-0.999, $p<0.01$). Possible systemic bias between the flow response and skin phantom absorption values is visualized in the residuals plot in Fig. 3(b). Here, differences in recorded flow values from the best fit are plotted as a function of phantom absorption value at each flow rate. A second linear fit of the residuals plot found no significant linear correlation between the absorption of the skin phantom and measured flow (R^2=0.025, p=0.29).

3.2. Clinical validation

Of 100 patients seen at the vascular clinic, a total of 167 limbs were measured with both toe pressure and the FlowMet device. Thirty-three limbs were excluded from the study due to amputation, metallic nail polish, or inability to secure the clip or pressure cuff. Of these, six were found to have low intensity below manufacturer limits, with five classified as White/Caucasian and one as Asian. Of 167 limbs included, 139 were classified as White/Caucasian, 12 as Asian, 23 as nonwhite Hispanic/Latino, and 3 as African American. For the binary stratification, 139 were classified in the lower melanin subcategory and 28 in the higher melanin subcategory. Flow and toe pressure values classified into lower- and higher-melanin subcategories are shown in a box and whiskers plot in Fig. 4. The 4-way demographic ANOVA test of toe pressure and flow values yielded an F-value of 0.89 ($p = 0.45$) and 1.31 (p=0.27), respectively. The 2-way ANOVA test between low- and high-melanin groups yielded an F-value of 0.4 ($p = 0.52$) and 3.68 (p=0.06), respectively. Both tests indicate that the variability of flow and toe pressures between demographic groups was not significantly different from the variability within the groups.

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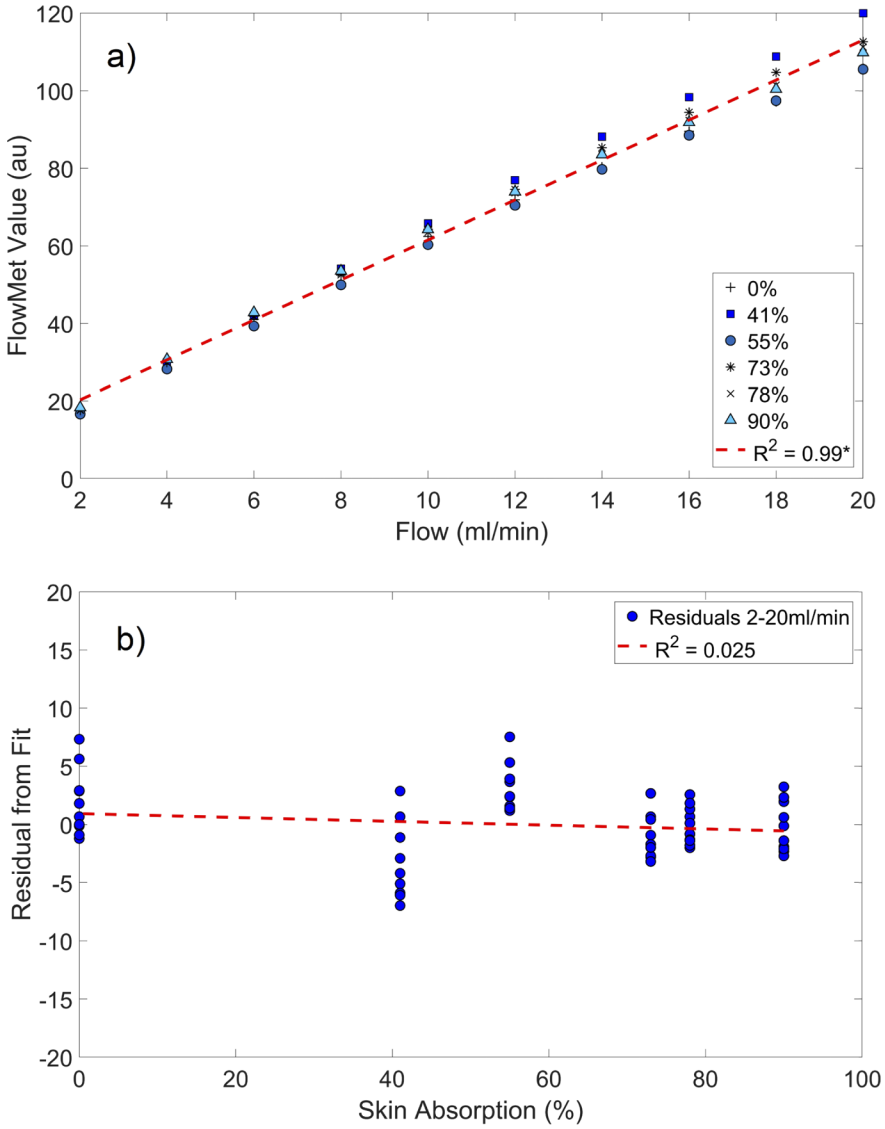

Fig. 3. Benchtop validation results. (a) SPG measurements (blue symbols) are plotted against known flow for skin phantoms of increasing absorption. The trendline (dashed red) demonstrated strong linearity ($R^2 = 0.99$). (b) Residuals plot of flow values (blue circles) as a function of skin phantom absorption show no significant linear trend (dashed red; $R^2 = 0.025$, p=0.29). *Indicates strong statistical significance (p<0.01)

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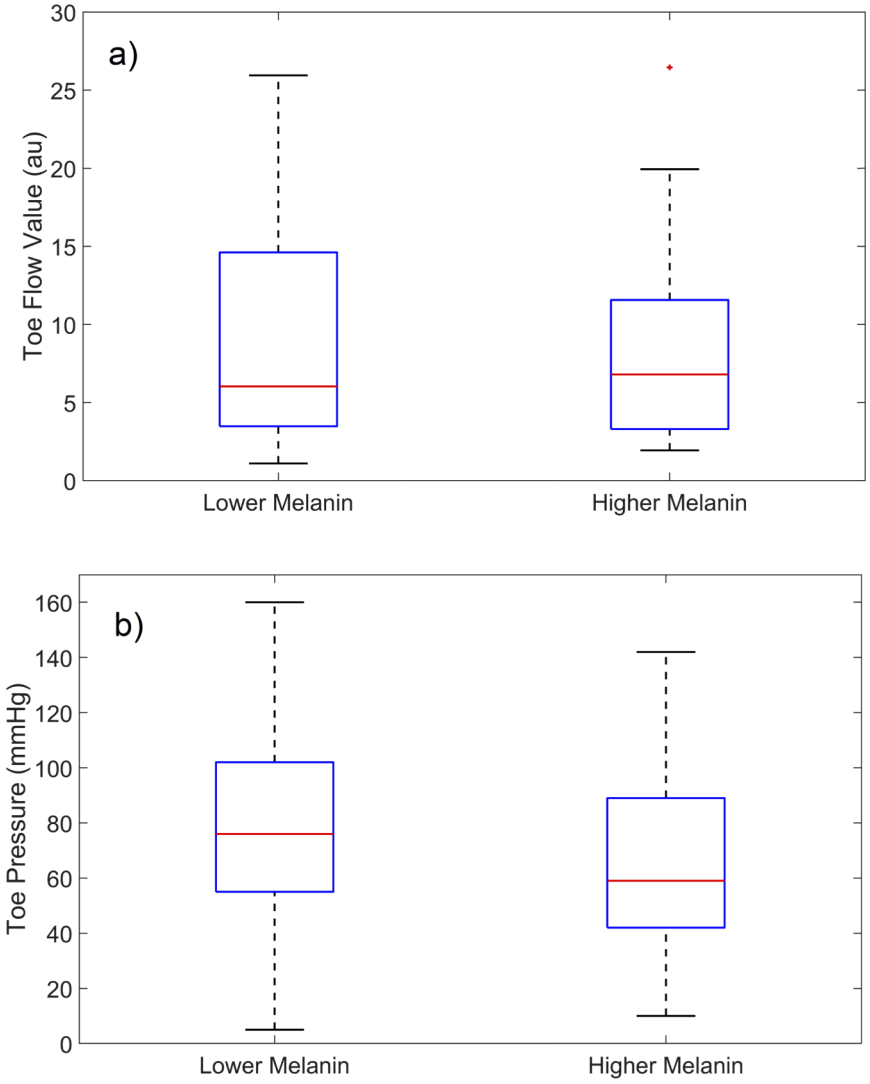

Fig. 4. (a) Box plot of the measured flow values from the low- and high- melanin subcategories showing significant overlap. (b) Box plot of the measured toe pressure values from the low- and high- melanin subcategories showing significant overlap.

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4. Discussion

Benchtop and in vivo studies were conducted to determine the impact of turbid skin layers with variable absorption on SPG signals. Because SPG relies on transmission LSI, all photons that enter the detector have necessarily traversed the dynamic layer. Therefore, contribution of static skin layers to the speckle contrast image are not necessarily expected assuming adequate diffusion. The empirical approach taken herein appears to confirm this assumption: static absorbing layers were not found to have a significant effect on the accuracy of SPG signals.

In the benchtop test where SPG was compared against known flow in the absence of a skin phantom, the observed trendline R^2 value was found to be nearly identical to prior literature (R^2=0.99 vs R^2=0.98) [15]. No decline in R^2 was observed with the addition of increasingly absorbing phantom layers, indicating a lack of additive effects on contrast or substantial changes in the signal-to-noise ratio due to added absorber. Note that transmitted intensity through the darkest layer remained above the specified minimum of 20 counts. Below this level, shot and dark noise may become a concern, and the SPG signal to noise ratio may be reduced.

No systemic effects in the response curve of SPG were observed when additional absorption was added to the skin phantom layers. Specifically, as shown in Fig. 3(b), no significant linear trend is seen between measured flow rate and skin phantom absorption. This suggests a lack of correlation between increasing or decreasing skin absorption and residual error. The figure does show possibilities of positive or negative bias in the residuals of individual phantoms. The authors speculate this may be due to slight variations in placement between data acquisitions, which highlights the importance of properly clipping the probe to ensure full volume sampling.

The in vivo study appears to further corroborate the benchtop validation study. Participants classified by estimated melanin concentration did not show any significant difference in mean SPG according to two ANOVA tests. Toe pressure values were used as a control against participant selection bias. However, both toe pressure and SPG signals did not indicate bias according to the ANOVA results. There may be two factors that work to reduce the bias of SPG signal in vivo. For one, drawing on the benchtop results, SPG measurements appear to be agnostic to the absorption of static layers. Second, prior studies have found the relative weight of melanin absorption on transmitted intensity is reduced as the thickness of the sample is increased. For tissue thicknesses greater than 3 mm, the impact of melanin becomes trivial compared to absorption effects from hemoglobin and photon scattering [27]. This may be supported by examining subjects where the signal intensity was overly attenuated. Five of six digits excluded from the study due to low intensity levels were classified in the lower melanin subcategory. While the sample is small it suggests that characteristics other than melanin contributed to this reduction, for example abnormal digit shape or improper clip placement.

Limitations of this study may include the validity of the benchtop model and the accuracy of the demographic classifications in predicting skin type. In future work, the digit analog and skin phantoms may be improved by including a smaller network of randomized flow that better simulate human microvascular structure. Additionally, in vivo studies may benefit from a more rigorous classification of skin melanin, such as the Fitzpatrick scale, and the intra-group variability of melanin could be evaluated at the measurement site [33]. The toe was chosen as the measurement site based on its clinical applicability for physicians treating PAD. However, the nail bed and plantar aspect of the toe may contain less melanin than other regions of the body. Therefore, an interesting avenue of future study would be to examine SPG transmitted through sun-exposed skin folds or other areas with high relative melanin content.

5. Conclusion

A benchtop experiment of skin-simulating phantoms demonstrated that SPG measurements from transmission laser speckle imaging are not significantly affected by static scattering layers with

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variable absorption. In vivo data of individuals with differing skin melanin content appeared to empirically corroborate benchtop results that SPG is not significantly affected by melanin absorption in a study of 167 human subjects.

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Disclosures

At the time the research was performed the authors were full-time employees of Laser Associated Sciences and are now employees of Medtronic.

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