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Hyperspectral imaging photoplethysmography: insights into the signals' origin and usage

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Abstract: This work explores the hyperspectral characteristics of imaging photoplethysmography (iPPG) to (1) deepen the understanding of iPPG signals' origin, and (2) assess iPPG's capacity to capture the peripheral perfusion. Our analyses are based on an experimental study employing a hyperspectral snapshot camera for iPPG and laser speckle contrast imaging (LSCI) at the forearm. We demonstrate a pronounced correlation between the wavelength-dependent normalized AC component from iPPG and the extinction coefficient of oxyhemoglobin ($r = 0.975$). Further, we show iPPG's ability to explain LSCI (linear mixed models' β up to 0.692). Considering the entire optical spectrum, our results support blood volume changes as a dominant factor for iPPG signals' generation and demonstrate iPPG's capacity to monitor peripheral perfusion.

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1. Introduction

Imaging photoplethysmography (iPPG) is a non-contact method to derive vital parameters from modulations of superficially reflected light [1,2]. The technique is very attractive because simple hardware, like common RGB cameras, can be used and its application is highly convenient. At the same time, iPPG contains a wide range of information and multiple vital parameters can be derived. The majority of works focuses on heart rate (HR) and heart rate variability (HRV) [3,4]. Beyond HR and HRV, respiration and arterial oxygen saturation by pulse oximetry (SpO_2) have been addressed [3–5]. Some works also focus on the superficial perfusion, where longitudinal variations, i.e., variations in perfusion strength over time and stimuli [6–8], as well as spatial variations [9–11] of perfusion were considered.

While the use of iPPG is growing and clinical applications are under debate [12,13], there are still concerns and an ongoing debate regarding the signals' origin. Very similar to contact PPG, blood volume changes have been assumed to be most relevant to signal generation in iPPG [14,15]. Kamshilin et al., in turn, described the tissue compression theory [16,17], which attributes iPPG's signals to pulse-pressure-driven mechanical compression of the dermis. With respect to applications beyond HR, particularly for iPPG-based SpO_2 , applications to assess perfusion and, more generally for all applications trying to analyze iPPG signals' shape beyond the cyclic variation with HR, a deeper understanding of the fundamentals of the technology is of great interest [15].

The primary objectives of this work are (1) to deepen the understanding of iPPG signals' origin, particularly to verify whether experimental observations are consistent with the blood volume theory and (2) to assess iPPG's capacity to capture peripheral perfusion. Both objectives have immediate relevance for future applications of iPPG. To this end, we derive the spectral characteristics of blood pulsation (spectral pulsation signature) using hyperspectral (HS) imaging and explore its behavior upon stimuli against laser speckle contrast imaging (LSCI). Experimental

data of our own, acquired using a unique technical setting, serve as base for our analyses. Our work fundamentally expands the scope of previous works on iPPG as multi- and hyperspectral analyses are not common [1]. While most works use RGB cameras, only few works use NIR wavelengths, consider multiple single wavelengths from visible range or use combinations of single wavelengths from visible and NIR range [18–20]. Our work extends previous works by capturing HS information and analyzing the acquired data in an innovative way.

The remainder of this work is organized as follows. In section 2, we outline the existing theories on the background of iPPG and provide a theoretical justification for the approach pursued in this work. In section 3, we detail the experimental study, employed data processing and statistical assessment. Section 4 provides results on the spectral characteristics of iPPG and the longitudinal behavior. Finally, section 5 and section 6 discuss our findings against the state of the art and draw conclusions, respectively.

2. Background

2.1. *iPPG signals' origin*

The exact origin of iPPG signals remains a subject of ongoing scientific debate, with research identifying three principal contributing mechanisms [15,21,22].

A first hypothesis attributes iPPG formation to cyclic changes in the orientation and deformation of red blood cells (RBC) driven by pulsatile flow, whereby the transition from diastole to systole causes erythrocytes to shift from random alignment to a configuration parallel to the vessel axis, thereby altering the optical attenuation through the tissue [23–26]. However, microscopic imaging studies of nailfold capillaries imply that RBC orientation changes and in turn modulates light in counter phase to the observed iPPG waveform [22]. The counter phase modulation is due to the light absorbance by RBC depending on their speed. In the systole, when the speed of the RBC is higher, light absorbance becomes lower than in the diastole. This mechanism may play a more prominent role at very shallow penetration depths, such as under blue light, where erythrocyte movement and aggregation dynamics can produce detectable light modulation [27,28].

The second, most widely accepted, model assumes that periodic blood volume variations in the dermal arterioles modulate tissue's optical absorbance with each heartbeat [14,27]. The model is based on the known behavior of light in tissue during volume fluctuations, including a well-developed and well-understood theory, particularly the modified Beer-Lambert Law. Monte Carlo simulations demonstrate that even lower wavelengths such as green wavelengths effectively probe pulsatile arterioles in the upper dermal plexus [14]. Video-capillaroscopic mapping likewise shows that the iPPG amplitude does not correlate with local capillary density, further supporting an arteriolar rather than capillary origin of the signal [14]. However, evidence from mechanically rigid tissue compartments such as brain, bone and dental pulp, where volumetric blood expansion is anatomically constrained, challenges a purely volumetric interpretation and supports oscillatory blood flow and RBC aggregation dynamics as complementary signal sources [26,29–31].

A third hypothesis proposes that pulse-pressure-driven mechanical compression of the dermis by underlying arteries reduces the spacing between superficial capillaries, thereby increasing the local capillary density and thus the absorption of light interacting with the capillary bed [16,17,32]. Kamshilin et al. motivated the hypothesis by the assumption that green light might not penetrate deep enough to interact with pulsating vessels but still produces the largest iPPG amplitudes of all wavelengths [17,33]. Kamshilin et al. also showed that the green wavelength iPPG's amplitude increases by up to eightfold when in contact with a glass plate, a magnitude difficult to reconcile with arterial blood volume changes alone [17,33]. The tissue-compression model offers a complementary explanation for the amplitude enhancement observed when skin is in contact with a glass plate [16,17]. Further support for the tissue-compression model

includes the observation of spatially adjacent skin regions exhibiting iPPG waveforms that are phase-inverted relative to one another, which is a finding that is difficult to explain with the blood volume variation model alone, since it would require the systole and diastole to occur simultaneously in neighboring tissue. The tissue compression model explains this through local variations in the mechanical coupling between arterial walls and the surrounding dermis [17,33]. On the other hand, the model faces important challenges from independent evidence. Over larger areas the number of capillaries is limited. If mechanical capillary compression were the sole or dominant mechanism at green wavelengths, local density increase in one location would lead to a decrease elsewhere and in an overall signal both sites would cancel each other out, which is not the case. Additionally, counter-phase iPPG waveforms have been attributed to ballistocardiographic artifacts (mostly superficial tilting owing to deeper pulsation) providing a very convincing explanation for phase inversion in iPPG signals [14,18]. Thus, counter-phase iPPG waveforms can be explained by either model, if ballistocardiographic artifacts are taken into account.

Notably, the aforementioned effects can coexist and even vary between measurement sites. However, in many cases there should be a dominant effect [15,27].

2.2. Theoretical foundation

Within this work we pursue a self-consistency check to deepen the understanding of iPPG signals' origin and, in particular, to verify whether experimental observations are consistent with the blood volume theory. Our starting point is the assumption that the blood volume theory applies to iPPG, i.e., blood volume changes are the most relevant mechanism for signal generation in iPPG. Under this assumption, some widely accepted simplifications, which are also crucial for pulse oximetry (SpO₂), apply [21]. These simplifications encompass constant path lengths and scattering losses during systole and diastole, a much larger direct current (DC) than alternating current (AC) component in the iPPG and an arterial oxygen saturation close to 100%.

The modified Beer-Lambert Law (MBLL) serves as background. According to the MBLL, the absorbance $A(\lambda)$ at a wavelength λ caused by a single absorber is given by

$$A(\lambda) = \ln \left(\frac{I_0(\lambda)}{I(\lambda)} \right) = \epsilon(\lambda) \cdot l(\lambda) \cdot c + G(\lambda) \quad (1)$$

with $I_0(\lambda)$ and $I(\lambda)$ input and attenuated intensities, $\epsilon(\lambda)$ the extinction coefficient of the absorber, $l(\lambda)$ the modified path length, c the concentration of the absorber and $G(\lambda)$ a scattering-dependent loss factor. If the difference in absorbance $\Delta A(\lambda)$ between diastole $A_d(\lambda)$ and systole $A_s(\lambda)$ is considered, only arterial hemoglobin needs to be taken into account as absorber. It holds

$$\begin{aligned} \Delta A(\lambda) &= A_s(\lambda) - A_d(\lambda) = \\ &= \epsilon(\lambda) \cdot l_s(\lambda) \cdot c_s + G_s(\lambda) - (\epsilon(\lambda) \cdot l_d(\lambda) \cdot c_d + G_d(\lambda)) = \\ &= \ln \left(\frac{I_0(\lambda)}{I_s(\lambda)} \right) - \ln \left(\frac{I_0(\lambda)}{I_d(\lambda)} \right) = \ln \left(\frac{I_d(\lambda)}{I_s(\lambda)} \right) = -\ln \left(\frac{I_s(\lambda)}{I_d(\lambda)} \right). \end{aligned} \quad (2)$$

Given $\Delta I(\lambda) = I_d(\lambda) - I_s(\lambda)$ and $I_d(\lambda) > I_s(\lambda)$, we can rewrite the term to

$$\begin{aligned} -\ln \left(\frac{I_s(\lambda)}{I_d(\lambda)} \right) &= -\ln \left(\frac{I_d(\lambda) - \Delta I(\lambda)}{I_d(\lambda)} \right) \\ &= -\ln \left(1 - \frac{\Delta I(\lambda)}{I_d(\lambda)} \right). \end{aligned} \quad (3)$$

With the Maclaurin series for $\ln(1-x) = -\sum_{n=1}^{\infty} \frac{x^n}{n}$ and $x = \frac{\Delta I(\lambda)}{I_d(\lambda)} \ll 1$ as $AC \ll DC$, we can omit higher-order terms of the Maclaurin series as they are negligibly small. The Maclaurin

series thus approximately simplifies to $\ln(1 - x) \approx -x$ and $\Delta A(\lambda)$ is then

$$\begin{aligned} -\ln\left(1 - \frac{\Delta I(\lambda)}{I_d(\lambda)}\right) &\approx \frac{\Delta I(\lambda)}{I_d(\lambda)} \\ &= \frac{I_d(\lambda) - I_s(\lambda)}{I_d(\lambda)} \\ &= \frac{AC(\lambda)}{DC(\lambda)}. \end{aligned} \quad (4)$$

$AC(\lambda)$ and $DC(\lambda)$ are the pulse amplitude and baseline from the iPPG signal at wavelength λ and the term can be understood as a spectral pulsation signature. The expression itself is part of the well-known ratio-of-ratios in SpO_2 . By additionally assuming the scattering losses $G_s(\lambda)$ and $G_d(\lambda)$ and path lengths $l_s(\lambda)$ and $l_d(\lambda)$ to be constant through systole and diastole, i.e., $G_s(\lambda) = G_d(\lambda) = G(\lambda)$ and $l_s(\lambda) = l_d(\lambda) = l(\lambda)$, ΔA is given by

$$\Delta A(\lambda) = \frac{AC(\lambda)}{DC(\lambda)} = \epsilon(\lambda) \cdot l(\lambda) \cdot (c_s - c_d) = \epsilon(\lambda) \cdot l(\lambda) \cdot \Delta c. \quad (5)$$

Equation (5) expresses a proportional relation between $\frac{AC(\lambda)}{DC(\lambda)}$ and extinction coefficient $\epsilon(\lambda)$ times path length $l(\lambda)$, i.e.,

$$\frac{AC(\lambda)}{DC(\lambda)} \propto \epsilon(\lambda) \cdot l(\lambda). \quad (6)$$

The extinction coefficient $\epsilon(\lambda)$ varies by almost two and a half orders of magnitude over the range of optical wavelengths (450 nm to 850 nm). For the path length $l(\lambda)$, in a restricted wavelength range a linear relation [34] and even over a large range much smaller variations can be assumed [21,27]. As $l(\lambda)$ is proportional to the differential path length factor (DPF) [21], we can estimate that $l(\lambda)$ varies by less than one order of magnitude in the dermis by using the tissue model by Jacques and the definition of the DPF by Scholkmann and Wolf [35–37]. Eq. (6) is thus dominated by $\epsilon(\lambda)$ and can be approximated by

$$\frac{AC(\lambda)}{DC(\lambda)} \propto \epsilon(\lambda). \quad (7)$$

As arterial hemoglobin is partially oxygenated, its extinction coefficient $\epsilon(\lambda)$ consists of two components, namely

$$\epsilon(\lambda) = SaO_2 \epsilon_{HbO_2}(\lambda) + (1 - SaO_2) \epsilon_{Hb}(\lambda) \quad (8)$$

where SaO_2 is the arterial oxygen saturation and $\epsilon_{HbO_2}(\lambda)$ and $\epsilon_{Hb}(\lambda)$ are the extinction coefficients of oxygenated and deoxygenated hemoglobin, respectively. In healthy subjects under resting conditions arterial oxygen saturation is close to 100%. It thus holds

$$\boxed{\frac{AC(\lambda)}{DC(\lambda)} \propto \epsilon_{HbO_2}(\lambda)}. \quad (9)$$

If the blood volume theory applies to iPPG, the spectral pulsation signature, i.e., the wavelength-dependent normalized AC amplitude, thus should be proportional to the wavelength-dependent $\epsilon_{HbO_2}(\lambda)$. This work aims to verify this expectation by own experiments and data analyses.

3. Materials and methods

3.1. Experimental study

Experimental procedure: Fig. 1 shows the experimental protocol. Optical recordings were taken from the forearm in supine position. Our protocol included two types of stimuli, namely

a cold pressure test (CPT) and application of a pharmacologically active substance. CPT is known to trigger an instantaneous systemic response mediated through the sympathetic nervous system. Most prominently, this response includes vasoconstriction and an associated rise of blood pressure [38]. In our experiment, CPT was executed twice employing custom instrumentation and a water temperature of approximately 1 °C. Participants were asked to keep their left hand submerged in the water for 20 seconds. Earlier removal was possible at any time if desired. CPTs took place right after a baseline recording (CPT1) and after the pharmacological stimulation was likely to have reached its full effect (CPT2).

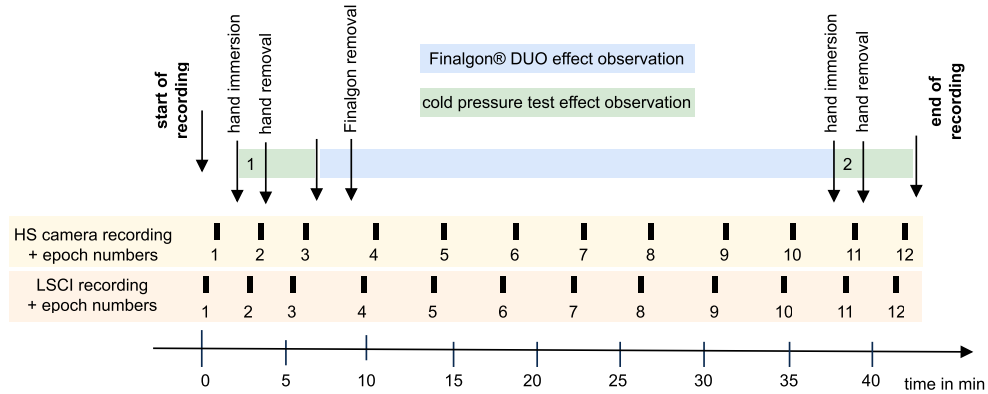


Fig. 1. Experimental protocol. We executed two CPT (1 and 2) and introduced a pharmacological stimulation by Finalgon ointment DUO. The ointment was removed after approximately two minutes to avoid specular reflections, but there is enough time to penetrate the skin barrier for the substance to sustainably affect the perfusion. Intermittent LSCI and HS camera recordings were done in 12 epochs j , numbered consecutively $j \in \{1, 2, \dots, 12\}$. Recordings had to be taken sequentially but we tried to minimize time in between and consider the physiological state to be the same for each pair of belonging LSCI and HS recordings. Each recording has a duration of 20 s per modality.

As a pharmacologically active substance, we used Finalgon ointment DUO. The ointment contains nonivamide (a capsaicinoid) and nicoboxil (a nicotinic acid ester). It provokes a local response characterized by local vasodilation and increased perfusion [6]. The ointment was applied to a predefined region, delimited by a tape which also serves to define the region of interest on the forearm. After applying the ointment, there was a two-minute exposure time. Then we removed any remaining ointment from the skin surface to minimize specular reflection caused by residue. This exposure time is enough for the active ingredients to penetrate the skin barrier and take effect even after superficial removal. To observe the effect of pharmacological stimulation, we monitored the reaction over approximately 35 minutes starting from ointment administration with the second CPT (CPT2) taking place approximately 31 minutes after administration.

Throughout the whole experiment, HS video recordings and LSCI recordings were done intermittently at the right forearm as indicated in Fig. 1. Recordings had to be taken sequentially but we minimized the time between both recordings. Each recording (HSI and LSCI) lasted approximately 20 s.

Hardware setting: Our hardware setting comprised two cameras (HSI and RGB), LSCI and instrumentation to record multiple biosignals. As not all hardware and signals are relevant to this work, we only describe the used components in detail. For HSI we used Cubert Ultris HR5 (Cubert GmbH, Ulm, Germany) with a spectral range from 450 nm to 850 nm. Videos were taken at a resolution of 290×275 pixels and a frame rate of 20 Hz. The HS camera was positioned approximately 45 cm above the forearm. HS measurements were taken perpendicular to the

measurement location. We employed indirect illumination by two halogen lights. As a reference measure for skin perfusion, we captured LSCI by PeriCam PSI NR (Perimed AB, Järfälla, Sweden). LSCI recordings were taken intermittently by rotating the measuring head perpendicular above the measurement location at a distance of approximately 25 cm. During the whole experiment we recorded multiple biosignals using Biopac MP36 (Biopac, Goleta, United States of America). Recorded biosignals covered electrocardiogram, (contact) photoplethysmogram and continuous non-invasive blood pressure. Synchronization between HSI and Biopac was done by feeding the flash trigger into Biopac. LSCI was not synchronized via hardware but assigned to specific times in the experimental protocol.

Participants: Overall, 17 healthy volunteers participated (5 female; age 32.6 ± 10.8 years). Skin tones covered fair to lightly-pigmented tone skin colors (Fitzpatrick Scale I - III according to qualitative, visual assessment). All participants gave written informed consent. The study was approved by the local ethics committee of the University of Augsburg (Zaunseder-30-10-2024).

3.2. Data analysis

Figure 2 shows the processing pipeline applied to HS video recordings and LSCI recordings. We analyzed data excerpts of 20 s per epoch.

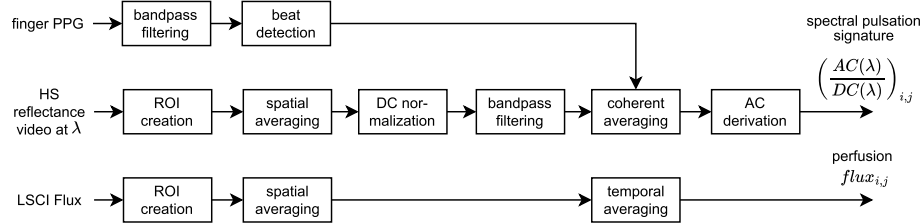


Fig. 2. Overview on the processing pipeline for a single subject and epoch. For each subject i we derive $(AC(\lambda)/DC(\lambda))_{i,j}$ and $flux_{i,j}$ per epoch j summing up to (at most) 12 assessments per subject.

HS data processing: Before processing HS data, raw HS data were pixel-wise transformed to reflectance by using a white-black reference. To further process the data, first we manually defined a region of interest (ROI) per epoch. Those ROIs were kept static throughout the 20 s of the epoch. Using the ROI, we extracted HS iPPG signals per wavelength by spatial averaging over the ROI and normalized the signals by the temporal average of the epoch (DC normalization). Notably, single epochs are assumed to be stationary. Using the temporal mean for DC normalization thus approximates a beat wise normalization approach as assumed in the theoretical derivation. Normalized HS iPPG signals were then filtered by a 4th-order Butterworth bandpass filter with cutoff frequencies of 0.5Hz and 8Hz. We used the finger PPG to detect single pulses and the median pulse interval PI . To yield a pulse template per epoch and wavelength, we employed coherent averaging of single pulses. To this end, for each detected pulse we segmented the interval $t_n - 0.1 \cdot PI$ to $t_n + 1.1 \cdot PI$, where t_n are the time instants of troughs in the finger PPG signal. We used the generated templates for qualitative assessment and we derived the normalized AC component $(AC(\lambda)/DC(\lambda))_{i,j}$ per subject i , epoch j and wavelength λ . Figure 3 illustrates the procedure to yield $(AC(\lambda)/DC(\lambda))_{i,j}$ and different intermediate results for one epoch.

The spectral pulsation signature was calculated by taking the mean of $(AC(\lambda)/DC(\lambda))_{i,j}$ over subjects i and epochs j (if such epoch was available for this subject) according to

$$\frac{AC(\lambda)}{DC(\lambda)} = \frac{1}{N} \sum_{i,j} \left(\frac{AC(\lambda)}{DC(\lambda)} \right)_{i,j} \quad (10)$$

The maximum number of intervals per subject, i.e., when no intervals are missing, is 12.

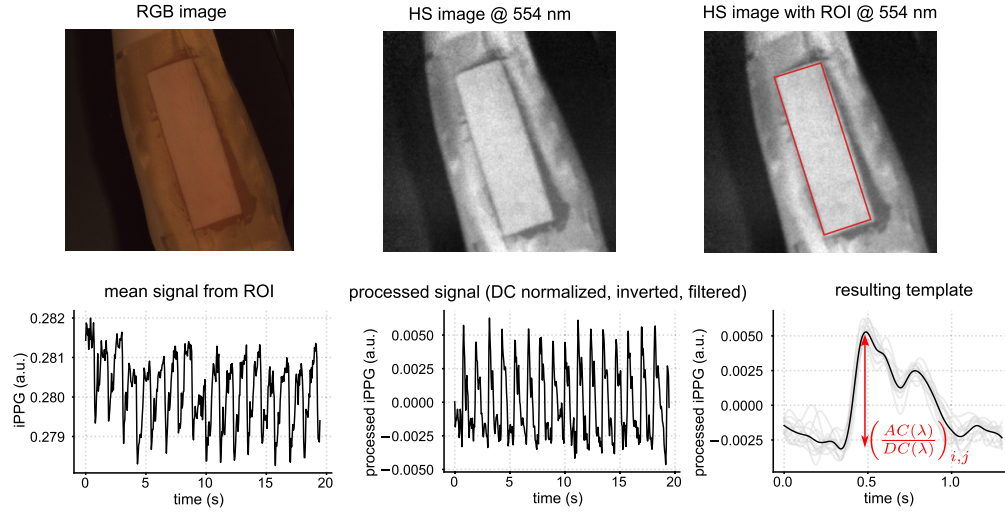


Fig. 3. Overview on the processing steps including intermediate results to yield $(AC(\lambda)/DC(\lambda))_{i,j}$. Shown here is subject $i = 4$ and epoch $j = 8$. Note that the RGB image has a slightly different view and is not used for processing but shown to illustrate the tape, which was used for a defined usage of ointment and manual ROI segmentation.

LSCI data processing: At first we manually defined an ROI per epoch j . Again, those ROIs were kept static throughout the 20 s of this epoch. LSCI *flux* was calculated by spatially and temporally averaging the pixel-wise flux values provided by the PeriCam PSI NR over the ROI to yield one value per epoch. No further processing was applied.

3.3. Statistical assessment

To compare the spectral pulsation signature $AC(\lambda)/DC(\lambda)$ with ϵ_{HbO_2} , and thus to assess Eq. (9), we first calculated the mean spectral signature across all epochs and subjects, resulting in a single representative spectrum across the 51 wavelengths. We then applied a non-parametric pairs bootstrap approach to the wavelength-dependent pairs of the mean spectral signature and ϵ_{HbO_2} . While this pairs-resampling scheme inherently disrupts the sequential autocorrelation between adjacent wavelengths, it provides a robust estimate of the correlation's sensitivity to individual spectral channels. For each bootstrap iteration, the correlation coefficient was calculated to construct the empirical distribution, from which the 95% confidence intervals (CI) were derived.

To assess the relationship of flux and the normalized AC component per wavelength we employed linear mixed effects models (LMM). For each wavelength λ , a separate LMM was fitted using restricted maximum likelihood. Both, LSCI flux and the normalized AC components from iPPG, were z-score standardized prior to modeling in order to yield directly comparable, unitless β coefficients, which represent the slope of the LMM (i.e., a standard deviation σ increase in flux is associated with a $\beta \cdot \sigma$ increase in the normalized AC component). They represent the population-average relationship between the explanatory variables for which the fixed effects are estimated and the response variable. LSCI flux was used as an explanatory variable. Subject was included as a random intercept to account for between-subject baseline differences and the repeated measures structure of the data, i.e.,

$$\left(\frac{AC(\lambda)}{DC(\lambda)} \right)_{i,j} = \beta(\lambda) \cdot flux_{i,j} + (b_0(\lambda) + b_i(\lambda)) + \epsilon_{i,j}(\lambda). \quad (11)$$

The random intercept consists of the population intercept b_0 and the subject-specific random intercept b_i . The residual is denoted by $\epsilon_{i,j}$. Standardized fixed effects estimates for flux β with their standard errors were extracted from each model (i.e., per wavelength). β coefficients were tested against zero using a two-sided t-test with a significance level of $\alpha = 0.05$. To control the false discovery rate across the 51 simultaneously tested wavelengths, i.e., to account for multiple testing, p values were adjusted using the Benjamini-Hochberg procedure [39].

4. Results

Recordings: Overall, 17 volunteers participated. Data of four subjects could not be used owing to persisting problems in recording and synchronization. From the remaining 13 volunteers, selected epochs could not be used for technical reasons (see Table 1). Overall, 146 single epochs could be used.

Table 1. Overview on missing epochs. One reason was a spontaneous outage of the HSI camera. The second reason was an obstructed view of the HSI camera by the LSCI system due to an overlooked position change. A third reason was movement because of pain during CPT. Four subjects were completely excluded because their recordings showed persistent/recurrent technical problems (like corrupted files and missing synchronization) resulting in less than 8 usable epochs

Subject	Excluded epochs	Reason
4	2, 9	HSI discontinuation
10	9	HSI view blocked
13	9, 10, 11, 12	HSI discontinuation
15	3	HSI view blocked
17	2, 11	Strong movements during CPT
2, 5, 7, 14	all	Persistent technical problems

Spectral pulsation signature: Fig. 4 shows the spectral pulsation signature $AC(\lambda)/DC(\lambda)$ according to Eq. (10) together with the extinction coefficient of oxygenated hemoglobin. The bootstrapped Pearson Correlation Coefficient between $AC(\lambda)/DC(\lambda)$ and $\epsilon_{HbO_2}(\lambda)$ is $r = 0.975$ ($CI = [0.957, 0.989]$).

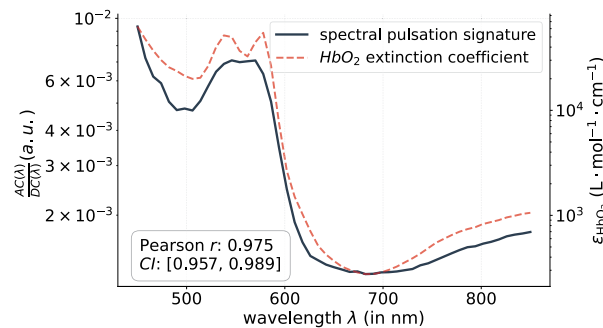


Fig. 4. The figure shows the spectral pulsation signature $AC(\lambda)/DC(\lambda)$ derived over all epochs and subjects together with the extinction coefficients of oxygenated hemoglobin. Extinction coefficients of oxygenated hemoglobin ϵ_{HbO_2} were taken from [40] (the values provided in [40] comply very well with a recent work of Gruensfelder et al. [41]). The graph visually demonstrates a high agreement between the spectral pulsation signature $AC(\lambda)/DC(\lambda)$ and the extinction coefficient of oxygenated hemoglobin, which supports blood volume changes to be a distinctive factor in iPPG signals' generation.

Perfusion assessment: Fig. 6 shows the temporal behavior of mean LSCI flux $flux_j$ and mean AC from iPPG ($AC(\lambda)/DC(\lambda)_j$) per epoch throughout the experiment. Both flux and AC, were standardized by their channel-wise means and standard deviations. The channel-wise parameters for $(AC(\lambda)/DC(\lambda))_j$ considered all wavelengths, usable subjects and usable epochs; for $flux_j$ we considered all usable subjects and usable epochs.

As expected, LSCI flux shows a marked and sustained response upon heat ointment. In addition, CPT intermittently increases perfusion slightly according to LSCI at $j = 2$ and $j = 11$.

With respect to HS iPPG, first of all, we noticed a distinct increase in iPPG's signal quality over the experiment. This increase is expected as signals from the forearm are known to show limited quality but improve with higher perfusion. Figure 5 exemplarily illustrates this effect by providing templates from different epochs j . The normalized AC also shows a reaction upon stimuli but the behavior varies between wavelengths. While the temporal behavior of blue and green wavelengths strongly resembles LSCI flux, red and infrared wavelengths differ in two aspects. First, the reaction upon heat ointment is much weaker. Second, CPT seems to cause a more pronounced decrease (epoch 2 and 11) followed by an increase after CPT (epoch 3 and 12). Figure 7 shows β per wavelength, i.e., the capacity of $(AC(\lambda)/DC(\lambda))_j$ to estimate LSCI flux $flux_j$. Most wavelengths (except a range of wavelengths around red wavelengths) show a significant relation as indicated by the colors in Fig. 7. However, even in Fig. 7 the varying correspondence of HS iPPG and LSCI becomes evident, e.g., for green light at $\lambda = 560$ nm the model yields $\beta = 0.692$ (variance explained by fixed effect $R^2 = 0.537$) while for red light at $\lambda = 680$ nm the model yields $\beta = 0.068$ (variance explained by fixed effect $R^2 = 0.010$).

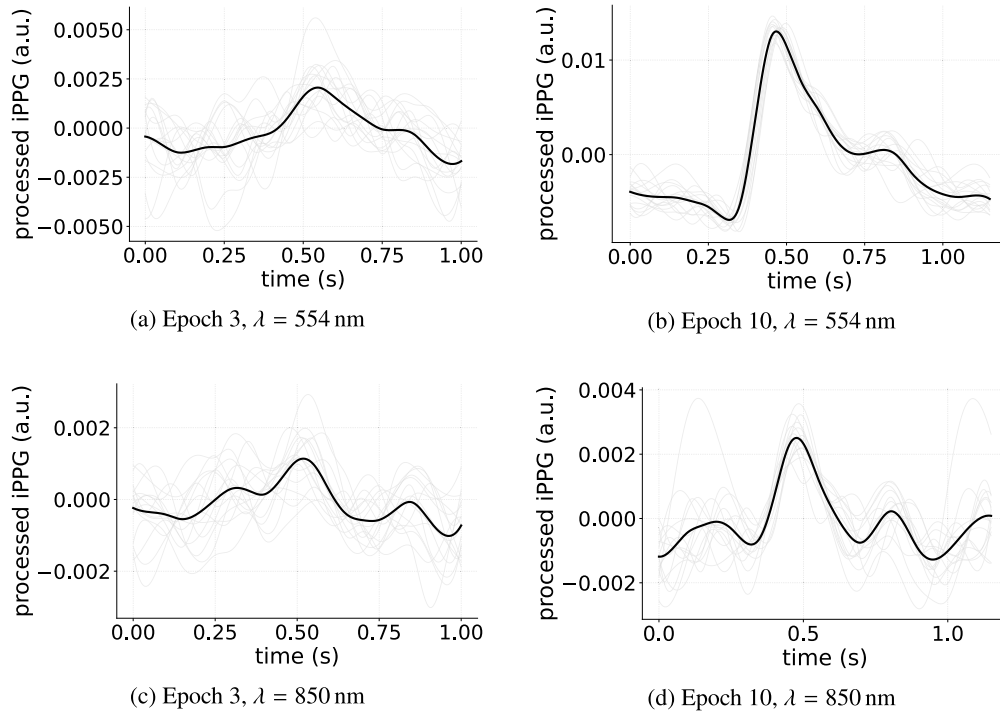


Fig. 5. Exemplary iPPG templates (and constituting single beats) at green wavelength ($\lambda = 554$ nm) and NIR wavelength ($\lambda = 850$ nm) from selected epochs for subject 9. Signal quality is typically low in epochs 1-4. After application of heat ointment (epoch 4), signal quality increases substantially in all wavelengths.

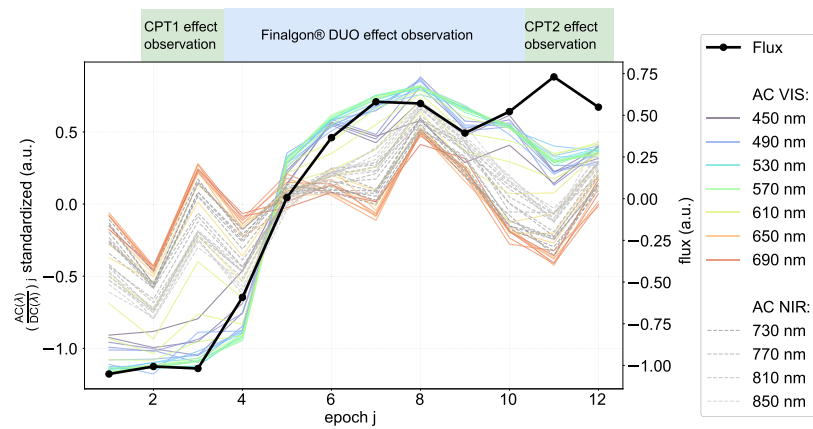


Fig. 6. Mean longitudinal behavior of $AC(\lambda)/DC(\lambda)$ and $flux$ for all wavelengths. The solid lines represent the visible wavelengths while the dashed lines represent the NIR wavelengths. For clarity the legend only refers to selected wavelengths. The graph visually demonstrates a high agreement between LSCI and iPPG at lower wavelengths with a maximum at green wavelengths. Higher wavelengths (red and infrared) show lower agreement and higher variation that is not contained in LSCI.

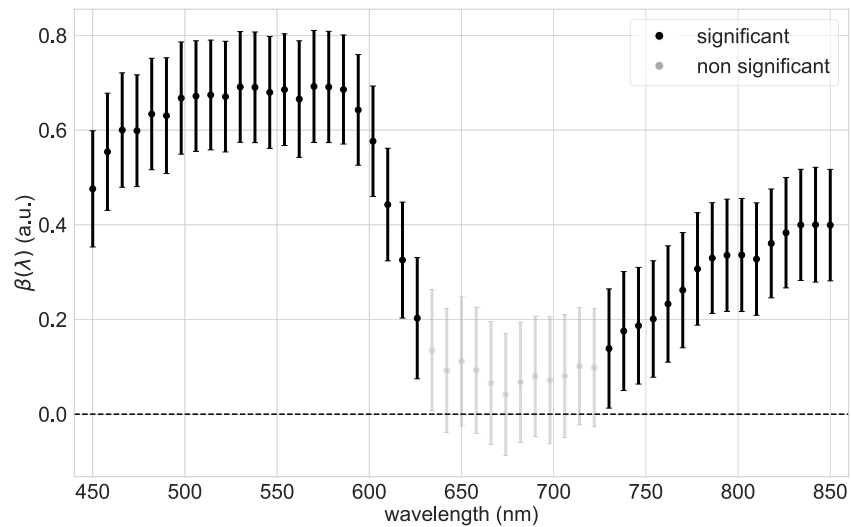


Fig. 7. Analysis of $AC(\lambda)/DC(\lambda)$ versus $flux$ by means of LMM. The figure shows the β per wavelength and the corresponding standard error. Significant and non significant channels are shown in black and gray colors, respectively. The high β coefficients in lower wavelengths with a maximum in green indicate iPPG's capacity to capture outcome changes and thus monitor perfusion.

5. Discussion

5.1. Spectral pulsation signature and iPPG background

As shown in Fig. 4, and also indicated by a high correlation coefficient of 0.970, the spectral pulsation signature matches very well the spectrum of the extinction coefficient of oxygenated hemoglobin. On an individual level, we observe even more details in the individual spectral pulsation signature, including the notch in extinction around 560 nm as shown in Fig. 8. However, taking the mean over subjects introduces blurring, e.g., due to varying hemoglobin characteristics, melanin concentrations and slight illumination differences. The simplifications we made in the derivation of equation 9 (e.g., the omission of $I(\lambda)$ in Eq. (7)) could also possibly explain some of the differences. Although some details vanish, the spectral pulsation signature fits the expectation on the behavior of pulsating arterial blood over the entire considered wavelength range. This finding supports the theory that blood volume variations are a crucial factor in iPPG's signal generation, which is in line with [14,15].

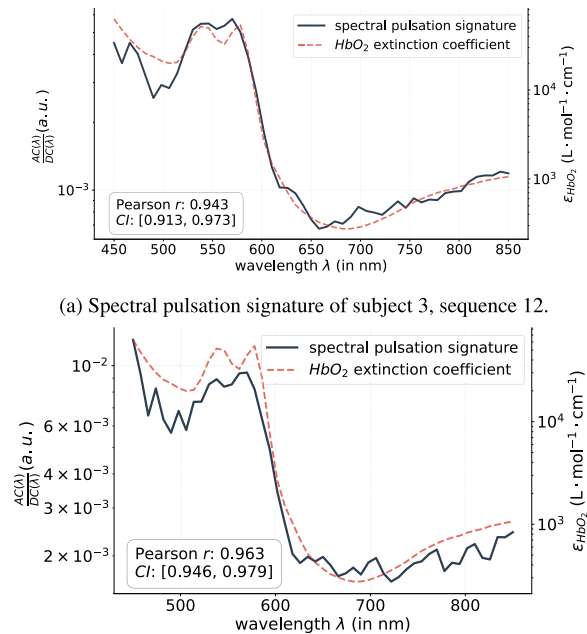


Fig. 8. Exemplary spectral pulsation signatures from two different subjects. Both graphs show the characteristic notch in extinction known from oxyhemoglobin. The graphs visually demonstrate a high agreement between spectral pulsation signatures $AC(\lambda)/DC(\lambda)$ and the extinction coefficients of oxygenated hemoglobin on an individual basis. However, together with the averaged spectral pulsation signature from before the graphs also highlight the blurring, which arises from intra- and interindividual averaging and is one limitation of our processing.

To what extent tissue compression, as it was introduced by Kamshilin et al. [16,17], plays an additional role is hard to distinguish (see also [42], which considers blood volume changes and tissue changes together) and cannot be answered by our experiment. This is especially true if one assumes that tissue compression also alters blood volume. In principle, the tissue-compression model is accompanied by blood volume variations in the capillary bed as the density of capillaries increases due to compression [22]. However, in our opinion the mechanical effect of tissue compression as the dominant effect would be likely to introduce additional effects (e.g., superficial deformation and associated reflectance variations) that deviate from Eq. (9) and from our results.

In addition, the ability to do pulse oximetry using green and red iPPG signals can also be seen as an indicator for the dominance of the effects explained by the blood volume theory [43]. In fact, when introducing their theory, Kamshilin et al. assumed an insufficient penetration depth for visible light to interact with pulsating vessels. Moco et al. have already shown in extensive simulations and associated experiments that visible light does interact with pulsating vessels [14]. This observation calls into question a fundamental assumption of Kamshilin's theory and favors volume changes as the signal's origin, as stated by [15].

Of course, one should not rule out additional effects to contribute to iPPG signals' generation. One should, in particular, be aware that dominant mechanisms might differ between wavelengths. The high $\beta(\lambda)$ at blue wavelengths might indicate such an additional effect. While blue wavelengths can reach the upper plexus they are not expected to be heavily affected by blood volume variations in the upper plexus. Blood cell aggregation as a mechanism contributing to iPPG signals' formation [27] and even tissue effects might be relevant. Even if capillaries do not pulsate, blood cell aggregation might result in the same effect as the pulsation of larger vessels, namely blood volume variations or more precisely an increase in effective absorber amount, this time at the capillary level and thus being integrated in our hypothesis. With respect to capillary blood volume variations, even capillary recruitment might contribute. In fact, pulsatile flow was shown to increase recruitment in lungs being at least a hint to suchlike effect [44]. More closely related, Gu et al. have shown intra-pulse-cyclic variations of hemodynamics at capillary level in retinal capillaries [45] but the relevance of such findings for iPPG remains to be explored in the future.

5.2. Peripheral perfusion assessment by iPPG

Our results show a pronounced relation between normalized AC ($AC(\lambda)/DC(\lambda)_j$) of iPPG and LSCI flux $flux_j$. This finding is in line with previous work combining both modalities [6–8]. While there are significant relations throughout the entire considered range of wavelengths, the differences between wavelengths are large. To some extent, differences (and the existence of significant changes or not) can be attributed to varying signal qualities between wavelengths. According to the literature and our data, green wavelengths show the highest signal-to-noise ratio. The higher quality makes it easier to show effects, which should be kept in mind in the interpretation of our findings.

As stated before, wavelength-dependent differences should be considered with respect to the stimuli.

Regarding the effect of heat ointment, green and blue wavelengths show marked reactions while red and NIR do not. This finding aligns well with other works like [46]. One reasonable explanation is the primary effect zone of the heat ointment, which we expect to be in the superficial plexus [47]. We assume blue and green wavelengths to be primarily affected by this region as well, which explains the strong reaction in these wavelengths. Red and NIR wavelengths are more affected by deeper vessels. These wavelengths show a reaction too, but this reaction is much less pronounced. This behavior can be well explained by the combination of the primary effect zone of the heat ointment and the assumed dominant regions of light absorption.

Even regarding CPT, there seem to be differences between wavelengths. In red and NIR wavelengths CPT provokes a more pronounced reaction than in shorter wavelengths even in CPT1, namely an instantaneous decrease in $(AC(\lambda)/DC(\lambda))_j$. A possible explanation again relates to the varying penetration depths of light and different contributions of vessels to iPPG signals' generation [14,27]. In general, CPT increases pulse pressure and vessel stiffness [38]. Concerning pulsatility, both effects are opposing but vessels are affected by varying degrees. The arterial vessels of the deep plexus, which we assume to play a significant role for red and NIR wavelengths, are larger and more muscular than those in the upper plexus [48]. Against that background, it is reasonable to assume, they are being affected stronger by vasoconstriction upon sympathetic

activation. Unlike in the superficial plexus, this increase might not be compensated by the increased pulse pressure, which might explain the reaction in higher wavelengths. Interestingly, at CPT2 we observe an effect even in lower wavelengths. This effect might be due to the fact that vessels are, owing to the active agent, initially at a dilated state making them more susceptible to vasoconstriction.

A final remark refers to a subtle but relevant and rarely discussed difference between LSCI and iPPG. Notably, LSCI indicates an increase in perfusion upon CPT. Despite vasoconstriction, such increase is reasonable owing to increased cardiac output and resulting flow velocities. Being strongly affected by pulsation, iPPG does not capture this increase but shows a decrease. Said difference is a result of different working principles and quantities that are acquired by both techniques (perfusion vs. pulsatility). In other words, iPPG, or more precisely the normalized AC component $(AC(\lambda)/DC(\lambda))_j$ which we used and is often used, can capture variation in skin perfusion but only in certain situations. Further methodological developments should try to complement $(AC(\lambda)/DC(\lambda))_j$ by additional parameters to be usable to monitor perfusion in more general terms.

5.3. Limitations

One obvious limitation of our work is the limited number of subjects and usable epochs. However, in our view, the results are quite clear with respect to the key points we made and we do not expect fundamental changes in these points from a larger cohort. Another limitation refers to the early epochs of our experimental data. As stated, such epochs exhibit poor signal quality and analyses there must be treated with caution as the pulse shape barely exists. With respect to handling HS data, refined procedures including separate consideration of varying melanin concentrations, ROI stabilization and improved template generation (e.g., accounting for intra-epoch heart rate variations and minor shifts for coherent averaging) might be possible, but again we believe that our key findings won't be affected. Finally, we want to emphasize that regions with vessels exhibiting strong pulsation and inhomogeneous regions are likely to exhibit additional or different effects. Signals acquired from such regions will always be difficult to interpret and might not be suitable for iPPG analyses beyond heart rate detection, to assess the perfusion.

6. Conclusion

To the best of our knowledge, our work is the first to demonstrate the HS characteristics of iPPG. We found a spectral pulsation signature which resembles the extinction coefficient of oxygenated hemoglobin to a high degree. Taking into account the theory provided in section 2, we interpret this finding as a strong indicator that, considering the entire optical spectrum, blood volume changes are distinctive for iPPG signals' generation. Even though we cannot rule out the influence of other mechanisms, like tissue compression, we can state that their effects are not strong enough to significantly undermine the central prediction that follows from the blood volume theory if it is valid.

With respect to using iPPG to monitor perfusion, our results demonstrate the capabilities of iPPG. As available methods for functional skin imaging, e.g., dynamic optical coherence tomography, laser Doppler and laser speckle imaging [49], are powerful but complex and more difficult to apply, we believe that iPPG has the potential to complement these techniques. However, our results also show iPPG's limitations and highlight wavelength-dependent differences. Such differences can be partially attributed to varying signal-to-noise ratios but they also seem to carry physiological information. A deeper understanding of the technique in general, of the existing wavelength-dependent differences and methods to exploit them are required to further develop iPPG towards routine use.

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Data availability. Data underlying the results presented in this paper are not publicly available at this time but may be obtained (concerns extracted iPPG and reference PPG data) from the authors upon reasonable request.

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