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ORIGINAL ARTICLE

A technique based on laser Doppler flowmetry and photoplethysmography for simultaneously monitoring blood flow at different tissue depths

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Abstract The aim of this study was to validate a non-invasive optical probe for simultaneous blood flow measurement at different vascular depths combining three photoplethysmography (PPG) channels and laser Doppler flowmeter (LDF). Wavelengths of the PPG were near-infrared 810 nm with source-to-detector separation of 10 and 25 mm, and green 560 nm with source-to-detector separation of 4 mm. The probe is intended for clinical studies of pressure ulcer aetiology. The probe was placed over the trapezius muscle, and depths from the skin to the trapezius muscle were measured using ultrasound and varied between 3.8 and 23 mm in the 11 subjects included. A provocation procedure inducing a local enhancement of blood flow in the trapezius muscle was used. Blood flows at rest and post-exercise were compared. It can be concluded that this probe is useful as a tool for discriminating between blood flows at different vascular tissue depths. The vascular depths reached for the different channels in this study were at least 23 mm for the near-infrared PPG channel (source-to-detector separation 25 mm), 10–15 mm for the near-infrared PPG channel (separation 10 mm), and shallower than 4 mm for both the green PPG channel (separation 4 mm) and LDF.

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1 Introduction

The measurement of blood flow at different vascular depths has several applications and has attracted the interest of several research groups. Studies have been performed using multichannel laser Doppler flowmetry [17, 30] as well as multilaser photoplethysmograpy [10]. Liebert et al. [17] attributed the depth discrimination ability to both wavelength and source-to-detector separation, whereas Tulevski et al. [30] and Gailite et al. [10] attributed it only to wavelength variations.

The assessment of blood flow has several important applications, such as during the healing of burn injuries [21, 29], transplantation of skin flaps [16], arteriogenesis [6] and investigations of pressure ulcers [20]; however, the methods need to be refined to also allow the discrimination of blood flow in different vascular beds.

Depth discrimination of tissue blood flow might help in investigating the aetiology of pressure ulcers, as ischaemia is considered to be a major parameter in this process. Today, the aetiology behind pressure ulcers is not fully understood and different theories exist. One implies that the ulcer starts superficially and develops downward, called top-to-bottom [5, 31], while another states that it starts next to the bony prominence and develops upward, called bottom-to-top [8]. These mechanisms might also co-exist. It is also important to consider deformation and tissue damage depending on mechanical load [4, 15, 28]. These theories are all based on tissue studies but use different body locations, subjects and even animal models, which could explain the discrepancies.

Thus, it is not clear at which depth, or depths, blood flow should be evaluated when investigating tissue viability regarding pressure ulcer aetiology. In general, tissue blood flow is not evaluated when the efficiency of different antidecubitus mattresses is investigated. Often, only the interface pressure between the body and the mattress, and the mattress's ability to redistribute this pressure, are taken into consideration [14]. One reason for this is the absence of a suitable measurement system enabling non-invasive measurements of blood flow in a realistic situation. Such a system needs to be placed between the patient and mattress and must not influence the blood flow or tissue.

To the authors' knowledge, no method combining photoplethysmography (PPG) and laser Doppler flowmetry, utilizing specific combinations of wavelengths and source-to-detector separations, has been established prior to the sensor probe used in this study first was used in measuring blood flow during pressure loading on healthy individuals [3].

The aim of the present study was to validate this non-invasive optical probe's ability to measure blood flows at different known vascular depths simultaneously. This validation study of the new optical sensor probe is an important step towards clinical patient studies.

2 Methods

2.1 Photoplethysmography

Photoplethysmography has been used mainly to non-invasively monitor skin blood flow especially during and after transplantation surgery. The technique is also used in pulse oximeters for monitoring arterial oxygen saturation. The PPG technique for non-invasive monitoring from deeper vascular compartments has been further developed by using an appropriate combination of optical wavelengths and distance between the light source (e.g., an LED = Light Emitting Diode) and the photo detector [18, 26]. A short distance between a green LED (560 nm) and a photo detector correlates to a short penetration depth due to the high absorption in blood (haemoglobin). On the other hand, a long distance between a near-infrared LED (810 nm) and a photo detector enables a deeper penetration depth, because the photons are absorbed to a lesser extent into the blood and are highly multiple-scattered in tissue (dermis, fat and collagen).

In PPG, light from a light source is directed towards the skin and is absorbed and scattered in the tissue. A small amount of this light is received by a photo detector placed, e.g., adjacent to the LED (reflection mode). Variations in the photo detector signal are related to changes in blood flow and blood volume in the underlying tissue. The PPG signal consists of an alternating current (AC) and a direct current (DC) component. The amplitude of the AC component depends on the pulsatile pressure as well as the pulsatile blood flow, pulsatile blood volume and the number of blood vessels in action for blood supply in a complex manner. The DC component of the signal varies slowly and reflects variations related to changes in total blood volume of the examined tissue, variations associated with, e.g., vasomotion, thermoregulation and respiration.

2.2 Laser Doppler flowmetry

Using laser Doppler flowmetry (LDF), the microcirculation in a small volume of tissue can be estimated. LDF is based on the principle that monochromatic light incident on the tissue is scattered and, if reflected by a moving scatterer, Doppler-broadened. This frequency shift is detected and presented in arbitrary units as an estimate of the perfusion. The perfusion scales linearly with the velocity (v_{RBC}) and the concentration of moving red blood cells (c_{RBC}), providing a low blood cell concentration to avoid multiple scattering [22–24]:

$$\text{perfusion} \propto \langle V_{RBC} \rangle \cdot C_{RBC}$$

The light is delivered to and from the skin by optical fibres. Laser Doppler techniques have been used extensively for tissue blood flow evaluation in different tissues and organs [24], including studies related to pressure ulcers [9].

2.3 Optical probe

The probe (Fig. 1) consists of a central photo detector, three pairs of LEDs and a laser Doppler fibre tip.

One pair of green LEDs (CR 10 SG, 560 nm) is situated at a distance of 4 mm on each side of the photo detector (BPW348) for measurement of the superficial blood flow. Two pairs of near-infrared LEDs (810-05AU, 810 nm) for monitoring deeper tissue blood flow are placed at a distance from the detector of 10 and 25 mm, respectively. The AC channels of the three PPG signals were named PPG_{Green}, PPG_{IR10} and PPG_{IR25}, respectively.

On one side of the detector between the two IR-LEDs, a fibre tip consisting of one sending and two receiving optical fibres with a separation of 1.1 mm are connected to a laser Doppler flowmeter (LDF, PeriFlux Pf2b, 632.8 nm, Perimed, Järfälla, Sweden), providing the LDF signal and measuring the most superficial blood flow. The optical components and fibre tip are embedded in a thin flexible black silicon sheet fixed in a 10 × 10 cm^2 wooden frame. Approximations made of the tissue volumes monitored by the different wavelengths are presented in Fig. 1b.

The probe was connected to a National Instruments DAQ-card 6062E (National Instruments, Austin, TX, USA)

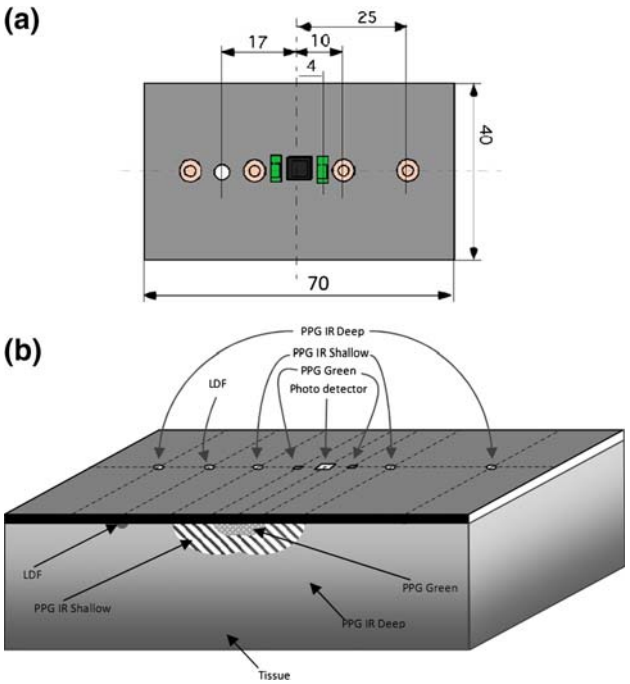

Fig. 1 **a** Schematic view of the probe; **b** comparison of approximated measuring volumes for each channel, presented as cross sections

and a LabView (National Instruments, Austin, TX, USA) program for data collection. The system sequentially pulses the different pairs of LEDs, and the backscattered light is collected by the photo detector and sampled into the measurement computer at a rate of 75 Hz. The program collects the LDF signal and AC and DC components from each of the three PPG signals.

In order to minimize the interference between the PPG signals and the LDF signal, optical filters were applied to the photodetectors of both the LDF and the photoplehysmograph. An interference filter cut from eye protection glasses (LaserVision, St. Paul, MN, USA) was placed over the PPG photodetector to reduce the influence of laser light (632.8 nm), and two heat transmitting mirror filters (Newport Corp, Franklin, MA, USA) were placed onto the LDF photodetectors to reduce the influence of both the green and the near-infrared light.

2.4 Ultrasound

The distance from the skin's surface to the fascia of the trapezius muscle was measured using an ultrasound Doppler (Vingmed system 5, GE Health, Oslo, Norway) 10 MHz linear probe at the position used for the blood flow measurement. The system allowed the visualization of a cross section of tissue 40 mm in width and 35–60 mm in depth.

The probe was fixed in a 10×10 cm^2 wooden frame replicating the physical dimensions of the optical probe, with a plexiglas window that made it possible to perform the ultrasound measurements using a similar physical interface area as during the blood flow measurements.

3 Measurement procedure and subjects

Eleven healthy subjects (six men and five women) with a mean age of 38 (range 22–58) years were recruited to participate in the measurement procedures. All subjects were informed about the project and gave informed consent to participate. The study was approved by the Research Ethical Committee at Linköping University, Sweden; Dno. M166–06.

The vascular depths reached by the different channels of the probe can be determined by stimulating the blood flow at a known tissue depth followed by measurements with all channels of the probe. By studying individuals with various known depth to muscle, the measurement range of the probe can be evaluated. A homogeneous blood flow was assumed in the whole trapezius muscle.

3.1 Measurement and provocation procedure

It is well known that blood flow increases significantly in muscles after both static and dynamic exercise [2]. A moderate contraction of the muscle should result in a local enhancement of the muscle circulation without leading to significant changes in the skin blood flow, although a strenuous contraction may eventually result in a skin blood flow increase due to physiologic thermoregulation [2]. In the present study, the blood flow was provoked through dynamic muscle contractions performed by the subject rhythmically lifting the right shoulder upward towards the ear (1 lift every 2–4 s) while grasping a dumbbell weighing 4–6 kg (women) or 7–10 kg (men). The exercise was designed by a physiotherapist to activate only the trapezius muscle. The trapezius muscle was chosen since it is known to be easily controlled voluntarily, a property considered to be of utmost importance in this study, even though this position of the shoulder not is considered to be especially prone to pressure ulcer development.

The trials were performed at a room temperature of 22–25°C. All subjects rested for 15 min in a sitting position before the trial began, to allow blood flow stabilization. In all subjects, blood flow was first measured for 60 s to establish the skin and muscle reference blood flow. Thereafter, the muscle contractions were performed for 40–60 s and post-exercise hyperaemia was monitored for 5 min.

A PPG recording is presented in Fig. 2 where the expanded views (in circles) show the signal before, during and after the provocation. During the exercise phase, most

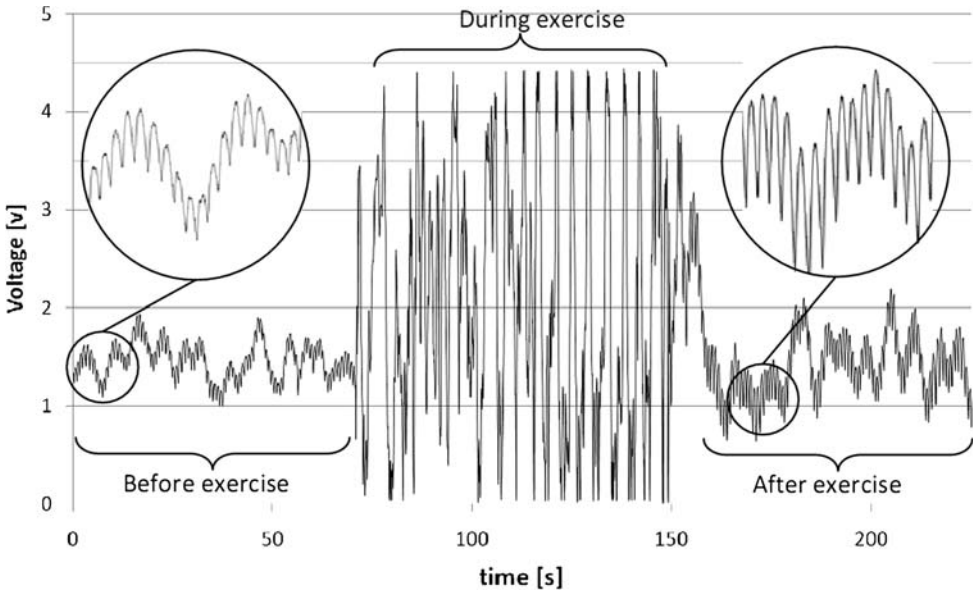

Fig. 2 PPG recording, before, during and after exercise. During the exercise the blood flow signal is hidden in the movement artefacts, and this part of the signal has not been evaluated

of the signal was rendered un-interpretable due to movement-induced interferences.

The ultrasound measurement was performed with the subjects sitting on a chair with relaxed muscles. A single cross-sectional image of the descending part of the trapezius muscle was visualized. The distance was read from this image (Fig. 3), and blood flow was measured directly afterwards. D_1 is the position on the skin's surface just beneath the plexiglas window, D_2 is at the exterior muscle fascia of the trapezius muscle, D_3 is at the fascia between the trapezius muscle and supraspinatus muscle, and D_4 is at the bone. The depth from the surface (D_1) to the muscle fascia (D_2) was measured at the middle and edges of the visualized area (P_1–P_3). A mean value was then calculated using the two highest readings. The depth from the surface (D_1) to the bone was measured at both edges of the visualized area (P_1 and P_3). The measured depths are presented in Table 1.

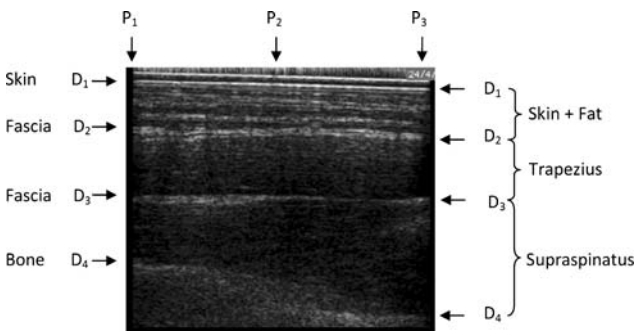

Fig. 3 Ultrasound image used to determine depth of muscle and bone

3.2 Data analysis

Blood flow was measured with LDF and the three PPG AC channels PPG_{Green}, PPG_{IR10} and PPG_{IR25}. The blood flows measured with the laser Doppler were represented as perfusion values, in arbitrary units (V) and calculated as a mean value. As the amplitude of the PPG-AC component depends on pulsatile blood flow, a custom-designed computer program, made in Matlab (the Mathworks, Natick, MA, USA), identified the peak-to-peak values of each PPG AC channel and provided the mean value (V), which is proportional to the blood flow in the tissue area under examination.

Blood flow was measured and averaged for 60 s before the exercise (rest) and for 5 min post-exercise using LDF and the three PPG AC channels. These averaging periods were chosen to ascertain accurate amplitude evaluations of the PPG signal [1]. A relative blood flow change of more than 10% was regarded as a reaction (Table 1).

To determine the vascular depths reached by the different channels, the ability to detect the expected increased blood flow post-exercise was evaluated in individuals with the trapezius muscle at various depths (D_2).

The Wilcoxon one-sided signed-rank test for paired data was used on each of the channels to determine whether there was a significant increase of the blood flow due to the provocation. The limit of 99% was regarded as significant.

4 Results

The relative change in blood flow post-exercise for all subjects is shown in Fig. 4 (left axis) together with the

Table 1 Classification of post-exercise hyperaemia based on the 10% limit for eleven subjects

Subject	Distance skin-fascia (D_1–D_2) (mm)	Distance skin-bone (D_1–D_4) (mm)	PPG_{IR25}	PPG_{IR10}	PPG_{Green}	LDF
1	3.8	12.5–16.2	Increase	Increase		
2	4.5	13.0	Increase	Increase		Decrease
3	8.2	nd	Increase	Increase		
4	9.3	22.5–26	Increase	Increase		
5	9.7	20.5–27	Increase	Decrease	Decrease	Decrease
6	12.2	nd	Increase	Increase		
7	13.3	nd	Increase			
8	14.5	32–35	Increase	Increase		
9	17	28.5–30	Increase			
10	19.8	nd	Increase			
11	23	38	Increase			
P value			<0.005	–	–	–

Significance levels calculated using Wilcoxon signed-rank test on paired data

nd not determined

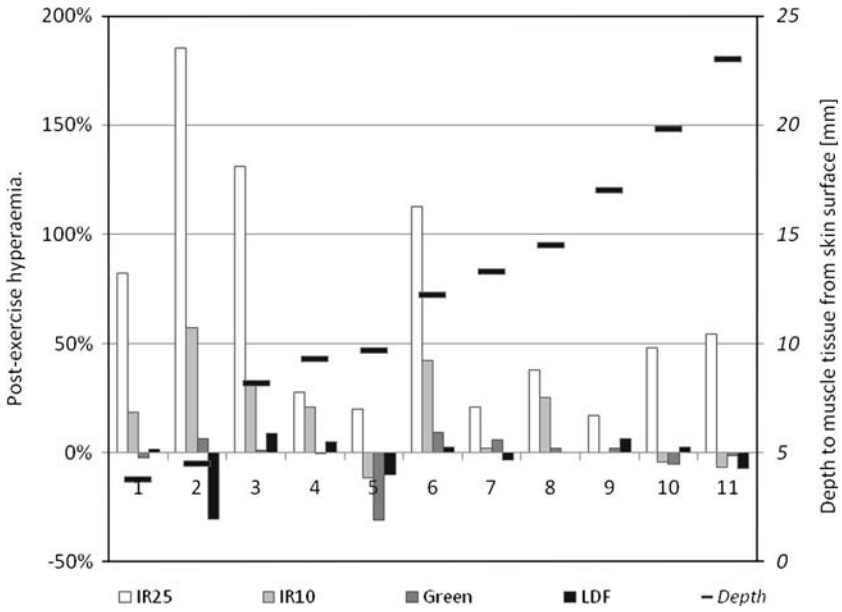

Fig. 4 Relative change in blood flow (*left axis*) after exercise related to measured depth between skin and muscle fascia in mm (*right axis*). All subjects showed an increase in blood flow for the deepest channel, PPG_{IR25}. For the second deepest channel, PPG_{IR10}, the four subjects with smallest distance between skin and fascia showed a post-exercise blood flow increase whereas one subject showed decreased blood flow. No subject showed a post-exercise increase in the PPG_{Green} or the LDF channel

distances between the skin and fascia of the trapezius muscle, D_1–D_2 (right axis). All subjects showed higher blood flow for the deepest channel (PPG_{IR25}). For the second deepest channel (PPG_{IR10}), the four subjects with shortest distance between skin and fascia showed a higher blood flow post-exercise together with the subject with a muscle depth of 12.2 mm and the subject with a muscle depth of 14.5 mm. One subject showed lower blood flow (muscle depth 9.7 mm), and the subject with a muscle depth of 13.3 mm together with the three subjects with the greatest muscle depths (17-23 mm) showed stable blood flow in a comparison of pre- and post-exercise. Thus, a break-point could be seen in this channel. No one showed higher blood flow post-exercise in the PPG_{Green} or the LDF channel.

The data from Fig. 4 is also presented in Table 1 with changes of post-exercise blood flow changes over 10% regarded as a reaction. Together with the reaction (increase or decrease), the distances between the skin and fascia of the trapezius (D_1–D_2) and the distance from the skin surface to the bone (D_1–D_4) is presented. There was an increase in PPG_{IR25} for all subjects. In the case of PPG_{IR10} there was an increase regarding short distances between skin and fascia (D_1–D_2) in Fig. 2, but only minor changes for longer distances. One subject showed decreased blood flow. In the shallow channels (PPG_{Green} and LDF), no post-exercise blood flow increase was present and two subjects showed a decrease in blood flow.

From these findings, the PPG_{IR25} was found to reach a vascular depth of at least 23 mm, since this was the greatest depth of the trapezius muscle that was measured among the test subjects. Since the exercise was designed to provoke only the blood flow in the trapezius muscle, the

signal from PPG_{IR25} was supposed to originate from the muscle. The PPG_{IR10} was found to reach a vascular depth of approximately 10–15 mm, since the limit for this channel to monitor the post-exercise blood flow increase among these subjects was between 9.3 and 14.5 mm.

Since no post-exercise blood flow increase could be recognized in PPG_{Green} and LDF, it could be determined that these channels reached a vascular depth less than that of the subject with the most superficial muscle, 4 mm.

The result of the Wilcoxon one-sided signed-rank test for paired data showed a significant change in blood flow in PPG_{IR25} at a significance level of 99.5%. No significant change in blood flow could be seen in the other channels.

5 Discussion

In the present study, a non-invasive optical probe combining three PPG channels and LDF has been validated regarding its ability to assess blood flows at different known vascular depths. The probe is intended to be used in the investigation of pressure ulcer aetiology, and as a tool for assessing antidecubitus mattresses. For this, it must be possible to place the probe between the patient and the support surface. This can be achieved with the present probe, but in order to not influence the blood flow or the tissue, a softer and more flexible probe is required. This validation study is an important step towards clinical patient studies assessing tissue viability while the patient is lying on a mattress. The results show that this probe, combining PPG and LDF for simultaneous blood flow measurements, can be used as a tool to evaluate blood flows at different tissue depths.

This probe has previously been used to evaluate the influence on blood flow of externally applied pressure in elderly individuals [3]. The pressures applied corresponded to 37.5 and 50.0 mmHg, respectively, which are clinically relevant levels when a subject is lying on a standard mattress [9]. The most common response found by Bergstrand et al. [3] was an increase in blood flow, but in some individuals a decrease was recognized. The probe did not seem to have any major influence on skin temperature in that study. To facilitate the interpretation of discrepancies between the different channels observed in that and future studies using the probe, the present study was designed. In this study, the tissue was not exposed to compression, which might have influenced the blood flow. Instead, a controlled model of muscle circulation enhancement at known depths aiming at revealing the vascular depths reached by the probe was applied.

Several attempts have been made to monitor different physiological parameters from deeper vascular beds in humans using PPG. Some research groups have shown that it is possible to, e.g., measure foetal oxygen saturation using pulse oximetry and PPG [33], and foetal heart rate using near-infrared PPG [11]. In the latter case, a wavelength of 900 nm was used with a source-to-detector separation of 40 mm and the distances between the maternal skin and the foetal skin was 40–50 mm. Other examples of applications using modality similar to that of the PPG technique are studying blood flow in the patellar bone [25] and monitoring blood flow variations in the tibial anterior and trapezius muscles [26, 27, 32]. The latter cases show that different optical PPG probes may discriminate between superficial skin blood flow using a wavelength of 560 nm and a source-to-detector separation of 3.5 mm and muscle blood flow using a wavelength of 806 or 880 nm with a source-to-detector distance of 10, 20 or 25 mm. From the consensus of experimental assessments in these studies, one may assume that pulsatile blood flow variations may be monitored from approximate depths of 2 mm using 560 nm, 8–10 mm using 810 nm (source-to-detector separation 10 mm) and a depth of 20 mm using 806 or 880 nm (source-to-detector separation 20–25 mm).

The present study indicates that vascular depths of at least 23 mm are reached by the PPG_{IR25} using a wavelength of 810 nm and a source-to-detector separation of 25 mm. The PPG_{IR10} (wavelength 810 nm and source-to-detector separation 10 mm) was found to reach a vascular depth of approximately 10–15 mm. Thus, the findings for this specific probe design indicate that somewhat deeper vascular structures are reached compared to previous studies using similar PPG configurations. However, this study was performed on a limited number of individuals, and the discrepancies are reasonably small.

In this study, the vascular depths reached by both PPG_{Green} and LDF were found to be shallower than 4 mm. However, LDF is known to have a more shallow measurement depth of <1 mm [23]. Monte Carlo simulations have been used to investigate different probe configurations and an increased fibre separation is known to give a larger depth. A probe configuration with a fibre centre separation of 700 μm gave a median sampling depth of 233 μm in skin [13]. Thus, a somewhat deeper penetration depth can be expected by the present probe, since a fibre centre separation of 1.1 mm was used.

The vascular depths reached by this probe seem to cover the range of interest of studies of pressure ulcer aetiology [7, 19].

It remains, however, to further assess the maximal vascular depth possible to be reached by PPG_{IR25}, as well as how shallow PPG_{Green} reaches in this probe design. This can be achieved by extended studies including subjects with larger and smaller distances between skin and muscle, respectively. In this study, the range of 3.8–23 mm was covered.

In this study, a 10% change in blood flow was set as the limit to regard a change as a reaction. In a sensitivity analysis, however, any limit between 8% and 17% gave the same pattern (increase or decrease, Table 1).

One subject showed an evident decrease in shallow blood flow (PPG_{IR10}, PPG_{Green}, LDF), which might be an effect of pooling the blood to the deeper tissue. In another subject, this effect was reflected only by the LDF measurements. Pooling of blood between different vascular beds has been observed and described in the literature; for a review see Hodges et al. [12]. This phenomenon has to be considered when evaluating differential vascularization in tissue during provocation.

6 Conclusion

From this study, we can conclude that this probe, combining three PPG channels and one LDF channel, can be used as a tool for discriminating between blood flows simultaneously at different tissue depths. The present probe might be an important tool in the process of studying the development of pressure ulcers as well as the effect of influencing factors in a controlled environment. The vascular depths reached for the different channels in this study were at least 23 mm for the near-infrared PPG channel (source-to-detector separation 25 mm), 10–15 mm for the near-infrared PPG channel (source-to-detector separation 10 mm), and more shallow than 4 mm for both the green PPG channel (source-to-detector separation 4 mm) and LDF.

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