# Comparison of NIRS, laser Doppler flowmetry, photoplethysmography, and pulse oximetry during vascular occlusion challenges

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**Abstract**

Monitoring changes in blood volume, blood flow, and oxygenation in tissues is of vital importance in fields such as reconstructive surgery and trauma medicine. Near infrared spectroscopy (NIRS), laser Doppler (LDF) flowmetry, photoplethysmography (PPG), and pulse oximetry (PO) contribute to such fields due to their safe and noninvasive nature. However, the techniques have been rarely investigated simultaneously or altogether. The aim of this study was to investigate all the techniques simultaneously on healthy subjects during vascular occlusion challenges. Sensors were attached on the forearm (NIRS and LDF) and fingers (PPG and PO) of 19 healthy volunteers. Different degrees of vascular occlusion were induced by inflating a pressure cuff on the upper arm. The responses of tissue oxygenation index (NIRS), tissue haemoglobin index (NIRS), flux (LDF), perfusion index (PPG), and arterial oxygen saturation (PO) have been recorded and analyzed. Moreover, the optical densities were calculated from slow varying dc PPG, in order to distinguish changes in venous blood volumes. The indexes showed significant changes ($p < 0.05$) in almost all occlusions, either venous or over-systolic occlusions. However, differentiation between venous and arterial occlusion by LDF may be challenging and the perfusion index (PI) may not be adequate to indicate venous occlusions. Optical densities may be an additional tool to detect venous occlusions by PPG.

Keywords: near infrared spectroscopy, laser Doppler flowmetry, photoplethysmography, pulse oximetry, noninvasive optical techniques, physiological monitoring, vascular occlusions

## 1. Introduction

The monitoring of blood flow, blood volume and blood oxygenation in tissues has played an essential role in clinical applications in the last decades (Lima and Bakker 2005, Sakr 2010). For instance, in reconstructive surgery or trauma medicine, the prompt identification of local changes in perfusion and oxygenation can alert clinicians before arise of serious complications that may be undetected by global macrocirculation parameters or clinical observations (Lima and Bakker 2005, Smit *et al* 2010, Scheeren *et al* 2012). In this context, optical modalities such as near infrared spectroscopy (NIRS), laser Doppler flowmetry (LDF), photoplethysmography (PPG), and pulse oximetry (PO) have played a crucial role, due to their noninvasiveness, safe nature and their ability to provide local assessment of the microcirculation (Lima and Bakker 2005, Sakr 2010).

NIRS is an optical technique used for the monitoring of tissue perfusion and oxygenation. Near infrared light is shone in tissues and light attenuations are then processed for the estimation of concentrations of oxygenated haemoglobin, reduced haemoglobin, total haemoglobin and tissue oxygenation (Pellicer and del Carmen Bravo 2011, Scheeren *et al* 2012). NIRS-measured parameters have been demonstrated to monitor effectively perfusion changes in brain and muscle microcirculation in surgery, shock and trauma medicine, and reconstructive surgery (Smit *et al* 2010, Pellicer and del Carmen Bravo 2011, Scheeren *et al* 2012).

LDF measures arterial blood flow in the skin by detecting the Doppler shift caused by moving red blood cells (RBC) (Choi and Bennett 2003). Laser light is applied to the skin and the Doppler shift caused by moving RBCs is processed to obtain a unit-less index widely known as Flux (Choi and Bennett 2003). The index is directly proportional to the product of both RBC concentration and flow in the sampled volume (Choi and Bennett 2003). Flux can be used as a direct or indirect trend indicator of changes in blood flow alterations in the microcirculation due to vasoconstriction/dilation or arterial/venous impairment, skin flaps and burns, or for the assessment of skin microcirculation in shock and diabetic patients (Yuen and Fend 2000, Choi and Bennett 2003, Lima and Bakker 2005).

PPG is a noninvasive optical technique in which light is applied to tissues and the detected light by a photodetector is characterized by a pulsatile component (ac) and a slow varying component (dc). The PPG waveform can be used for calculation of several parameters such as heart rate, pulse transit time (PTT), noninvasive blood pressure, and respiration (Allen 2007, Shelley 2007). Measurement locations are usually extremities such as finger and earlobe, but several different locations such as the esophagus, splanchnic organs, and flaps have been investigated as well (Kyriacou 2006, 2013). While the ac component of the signal is predominantly used to assess local arterial blood inflow, the dc component has been proved to provide information on venous blood, respiration, vasodilation/constriction, blood pressure, and perfusion (Shelley 2007, Reisner *et al* 2008, Walton *et al* 2010, Abay and Kyriacou 2015). However, the main application of PPG signals is the noninvasive estimation of arterial oxygen saturation ($SpO_2$). The technique is commonly known as PO and relies on the direct relationship between the ratios of ac and dc PPG components at red and infrared wavelengths and $SpO_2$ (Kyriacou 2006, Allen 2007). An emerging application of PPG signals is the calculation of the perfusion index (PI) (Lima and Bakker 2005). The parameter is calculated as the ratio of the ac component over the dc component (ac/dc) and is believed to assess the adequacy of perfusion (Lima and Bakker 2005).

NIRS, LDF, PPG, and PO have been investigated singularly or in pairs, during a vascular occlusion test (VOT), in their ability to indicate changes in perfusion, oxygenation, or to differentiate between healthy and diseased (Galla *et al* 1999, Yuen and Fend 2000, Hallock and Rice 2003, Jarm *et al* 2003, Klaessens *et al* 2003, Gomez *et al* 2008, Bergkvist *et al* 2015). However, the results from singular studies are scattered due to different studies methodologies, applications, or populations investigated. To our knowledge, there has not been an attempt to investigate all four techniques simultaneously on healthy volunteers during different VOTs. Hence, the aim of this study is to investigate simultaneously the four modalities during two different VOTs. Furthermore, the sensitivity of the techniques on detecting changes in perfusion was assessed by applying different steps of vascular occlusions. NIRS, LDF, PPG, and PO sensors were attached on the left forearm and fingers of nineteen healthy volunteers and their responses during two different VOT protocols were analyzed.

## 2. Material and methods

### *2.1. Measurements system and signal acquisition*

In this study, an NIRS monitor (NIRO 200NX, Hamamatsu Photonics, Japan) was used to measure tissue oxygenation index (TOI) and normalized tissue haemoglobin index (nTHI). The instrument employs spatially resolved spectroscopy (SRS) (Suzuki *et al* 1999), and uses light emission wavelengths at 735, 810 and 850 nm, and silicone photodiodes for the estimation of changes in oxygenated haemoglobin, reduced haemoglobin, total haemoglobin, TOI, and nTHI. TOI is calculated as the ratio between absolute concentration of oxygenated and total haemoglobin (Suzuki *et al* 1999). The nTHI is calculated as changes in total haemoglobin concentrations and is normalized to the initial baseline value. An inter-optodes distance of 4 cm was utilized for deep penetration of light in tissue.

An LDF monitor (moorVMS-LDF2, Moor Instruments, UK) was used to measure blood flow in the skin. The device employs fibre optics at a separation distance of 0.5 mm and laser light with peak emission at 785 nm. The Doppler shift from RBC is measured from the detected light and the blood flow is expressed as Flux, a unit-less index.

A commercial pulse oximeter system (Radical 7, Masimo Corp.) was used to measure noninvasively $SpO_2$. Finally, a custom-made research PPG system (*ZenPPG*) was used to collect raw dual-wavelength PPG signals from the finger (Rybynok *et al* 2013). A finger reflectance PPG probe was designed and manufactured for acquisition of signals from the *ZenPPG*. The sensor was enclosed in a pulse oximeter finger clip and it comprises of two infrared LEDs (KP-2012SF4C, Kingbright, Taiwan), two red LEDs (KP-2012SRC-PRV, Kingbright, Taiwan), and a silicon photodiode (TEMD5010X01, Vishay Intertechnology Inc., USA). The red and infrared LEDs had a peak emission wavelength of 660 and 880 nm respectively. All the signals from commercial and custom-made devices were acquired on LabVIEW (National Instruments, USA) and digitized by two 16-bit Data Acquisition Cards (NI-PCIe6321, National Instruments, USA) at a sampling frequency of 400 Hz. The developed software was designed to acquire, display, and save the physiological measurements in real time.

### *2.2. Investigation protocol*

Nineteen (19) healthy subjects (12 males and 7 females; mean age: 31.05 $\pm$ 7.07 SD) were recruited for the investigation. Ethical approval was gained from the Senate Research Ethics Committee at City University London. Subjects with a history of cardiovascular disorders were excluded from the study. Measurements were taken at a room temperature of 23 ($\pm$1) °C

and each subject was seated on a comfortable chair. After measuring the participant's blood pressure, their arm was rested on a pillow whereas a blood pressure cuff was connected to a sphygmomanometer and placed around the left upper arm for induction of vascular occlusions. An LDF sensor was positioned on the left forearm, distal from the cuff and attached on the skin by a ring-shaped double-sided adhesive. The NIRS sensor was located above the left brachioradialis, distal to the LDF sensor, and fixed by means of double-sided clear adhesive tape. The custom-made PPG and commercial pulse oximeter sensors were positioned respectively on the second and third digit of the left hand.

The investigation consisted of two measurement protocols:

- Protocol 1: baseline measurements were acquired for 5 min followed by a 2 min venous occlusion at 60 mmHg. The cuff pressure was then released for 2 min before being re-inflated for additional 2 min at 20 mmHg exceeding the volunteer's systolic pressure. The cuff was finally released until all the signals returned to their baseline values.
- Protocol 2: 5 min baseline measurements were followed by seven intermittent occlusions at 20 mmHg, 40 mmHg, 60 mmHg, 80 mmHg, 100 mmHg, volunteer's systolic pressure, and total occlusion (20 mmHg over volunteer's systolic pressure). Each occlusion lasted for 1 min and it was followed by 1 min of recovery period when the pressure was released. After the last occlusion (total occlusion), measurements continued until the signals returned to baseline values.

Participants rested for a minimum of 10–15 min between the two different protocols in order to allow a full haemodynamic recovery. The maximum cuff inflation time was 5 s, whereas complete deflation time did not exceed 3 s.

### *2.3. Data analysis and statistics*

Post-acquisition analysis was performed in MatlabR2013a. TOI, nTHI, and Flux signals were filtered by a zero-phase low-pass filter (cut-off frequency: 500 mHz). The ac PPG component was obtained from raw PPG signals by applying a zero-phase band-pass filter (band-pass frequencies: 0.5–7 Hz) whereas the dc PPG component was obtained with a low-pass filter (cut off frequency: 500 mHz). the PI was estimated in a 2 s rolling window and was calculated as the ratio between pulsatile ac and dc PPG components of the infrared wavelength (see equation (1)). $SpO_2$ was calculated in a 3 s rolling window by applying a linear equation relating the ratio between ac and dc PPG components at both wavelengths (see equation (2)). In order to compensate for inter-subject variability, Flux and PI were normalized to the first 10 s of baseline measurements. Optical densities from red wavelength finger PPG signals were calculated as the natural logarithm of the ratio between dc PPG at baseline and dc PPG throughout the protocol (see equation (3)). Linear trends were then removed from optical densities by extracting a least-square fit on the data. The slopes of TOI, nTHI, and $OD_R$ during the vascular occlusions were used to express dynamic changes in these signals. The slopes were computed as the slope of the regression line on the data during each occlusion, and they were expressed as % $min^{-1}$ for TOI or A.U. $min^{-1}$ for nTHI and $OD_R$. The changes in parameters were expressed as mean of means ($\pm$SD). In order to test statistical difference between baseline and occlusions measurements, baseline values were calculated as the average of 1 min baseline segment, whereas occlusion values were estimated as the mean during the last 30 s of each occlusion. Wilcoxon signed rank test was used to assess statistical significance between different occlusions. A value of $p < 0.05$ was considered satisfactory for statistical significance.

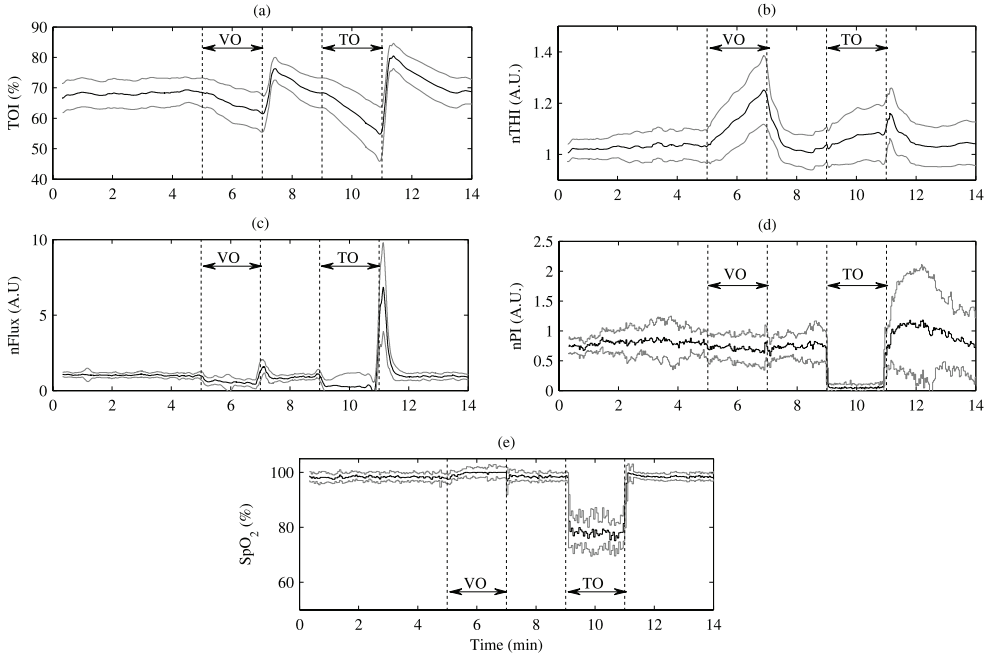


**Figure 1.** Mean changes in TOI (a), nTHI (b), nFlux (c), nPI (d), and $SpO_2$ (e) during protocol 1. Black traces: mean values among the volunteers. Grey traces: ±SD. Vertical dotted lines represent the duration of the occlusions. VO: venous occlusion. TO: total occlusion. $SpO_2$ fails during TO.

$$\mathrm{PI} = \frac{\mathrm{ac_{IR}}}{\mathrm{dc_{IR}}} \times 100 \tag{1}$$

$$\mathrm{SpO_2} = 110 - (25 \times R);\ \text{Where}:\ R = \frac{\mathrm{ac_R}/\mathrm{dc_R}}{\mathrm{ac_{IR}}/\mathrm{dc_{IR}}} \tag{2}$$

$$\mathrm{OD_R}(t) = \ln\left(\frac{\mathrm{dc_R}(0)}{\mathrm{dc_R}(t)}\right) \tag{3}$$

## 3. Results

### *3.1. Protocol 1*

Figure 1 shows the mean changes in protocol 1 in TOI, nTHI, normalized Flux (nFlux), and normalized PI (nPI) for the subjects investigated. Baseline values for TOI, nTHI, nFlux and nPI were respectively $68.10 \pm 4.51$, $1.02 \pm 0.05$, $1.00 \pm 0.14$, and $0.83 \pm 0.21$. During venous occlusion, venous blood pooling caused TOI, nFlux, and nPI to drop to $63.37 \pm 5.33$ ($p < 0.001$), $0.44 \pm 0.27$ ($p < 0.001$), and $0.69 \pm 0.25$ ($p = 0.03$) respectively, while nTHI increased to $1.22 \pm 0.12$ ($p < 0.001$). During total occlusion, TOI, nFlux, and nPI respectively decreased to $56.39 \pm 7.52$ ($p < 0.001$), $0.21 \pm 0.71$ ($p = 0.001$), and $0.06 \pm 0.06$ ($p < 0.001$). A small increase in ntHI was noticed during total occlusion ($1.09 \pm 0.09$, $p = 0.005$). During venous occlusion, the mean commercial $SpO_2$ dropped significantly

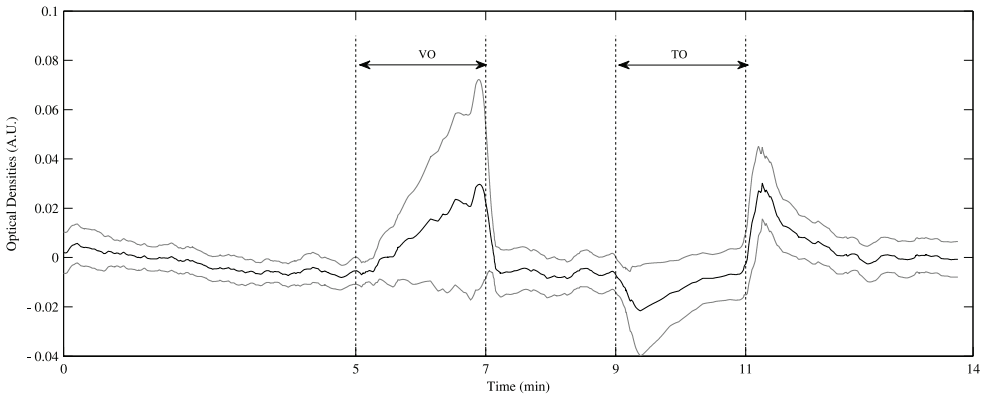


**Figure 2.** Mean changes in optical densities estimated from finger red PPG during protocol 1. Black trace: mean. Grey traces: ±SD. Vertical dotted lines represent the duration of the occlusions.

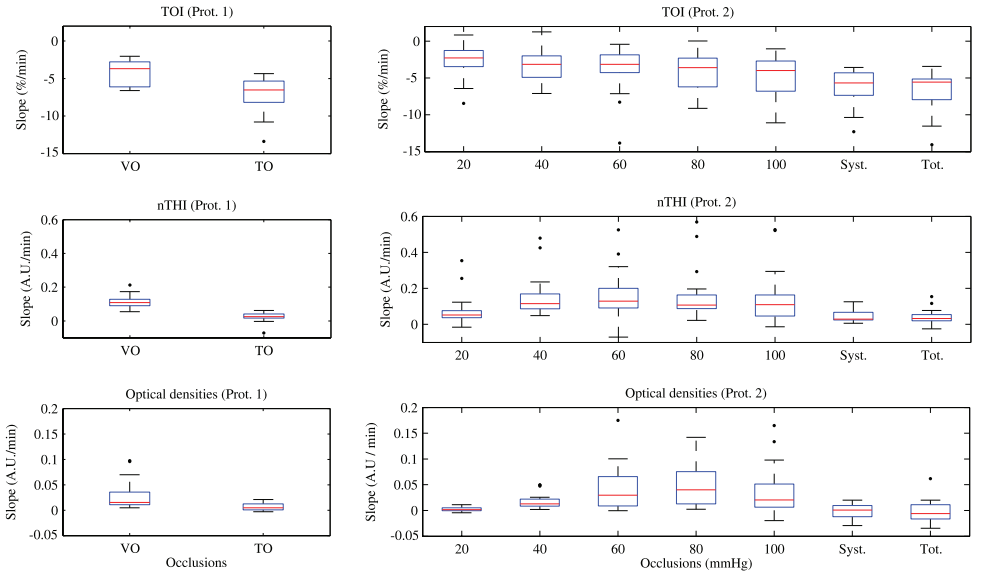


**Figure 3.** Slope changes during protocol 1 and protocol 2 for TOI, nTHI, and Optical densities. Prot. 1: protocol 1. Prot. 2: protocol 2. VO: venous occlusion. TO: total occlusion.

from a baseline of 98.91 ± 1.06 to 97.85 ± 1.68 ($p < 0.001$), while in total occlusion the pulse oximeter failed on providing $SpO_2$ values. However, the $SpO_2$ estimated from raw PPG signals exhibited a non-significant increase ($p = 0.06$) throughout venous occlusion and failed during total occlusion. Due to the absence of arterial pulsations, both commercial pulse oximeter and custom-made PPG device failed to provide reliable values of $SpO_2$ during oversystolic occlusion pressure.

The results from the optical densities estimated from PPG from the finger are showed in figure 2. Red wavelength optical densities consistently increased during venous occlusion ($p = 0.005$) whereas decreased during total occlusion ($p < 0.001$).

Figure 3 shows the changes in slopes calculated from TOI, nTHI and optical densities during protocol 1. The TOI showed a steeper desaturation during total occlusion than venous occlusion ($p < 0.001$). The slope of nTHI during venous occlusion was significantly higher compared to total occlusion ($p < 0.001$). Similarly, the positive slope of the optical densities during venous occlusion indicated an increase in light attenuation due to venous engorgement, whereas it was nearly nil during total occlusion.

### *3.2. Protocol 2*

Table 1 shows the means and *p*-values of TOI, nTHI, nFlux and nPI during protocol 2. TOI demonstrated deoxygenation during all the occlusions due to venous engorgements or total occlusions. The nTHI, which expresses the blood volume changes in tissue, gradually increased from baseline until a maximum venous engorgement was reached between 40 mmHg and 100 mmHg. Systolic and total occlusions (venous and arterial) produced a less pronounced increase in in nTHI due to venous and arterial occlusion. NFlux decreased during the occlusion at 20 mmHg, and it showed very similar values throughout occlusions between 40 and 100 mmHg. During systolic and total occlusion, nFlux dropped reaching the *biological zero*. Significant changes in nPI during protocol 2 were noticed only after occlusion exceeding 40 mmHg, whilst occlusion at 20 mmHg did not produce any significant change. Baseline $SpO_2$ from both commercial and custom-made pulse oximeters were $97.82 \pm 1.05$ and $97.29 \pm 2.76$ respectively. Consistent $SpO_2$ changes have been taking place only after occlusion at 100 mmHg ($96.88 \pm 30.52$ ($p = 0.001$) and $95.72 \pm 9.61$ ($p = 0.001$) for both commercial and custom-made respectively), while during previous occlusions the $SpO_2$ did not change noteworthy. During systolic and over-systolic occlusion pressures, the pulse oximeters failed and did not provide reliable $SpO_2$ values.

The optical densities in protocol 2 showed changes in blood volume due to vascular occlusions. As presented in figure 4, significant changes from baseline were observed only for venous occlusions exceeding 40 mmHg. Maximal changes were at 80 mmHg, showing complete venous occlusion and resultant blood engorgement. Optical densities dropped in both systolic and over-systolic pressures.

During protocol 2, the TOI desaturation slopes in the occlusions between 20–80 mmHg were not different ($p > 0.05$), whereas significant slope changes were only observed between the venous occlusions (i.e. 20–80 mmHg) and the occlusions exceeding 100 mmHg ($p < 0.003$). Similarly, the nTHI slopes during 40–100 mmHg were not different between each other ($p > 0.05$). A change of slope in nTHI was only taking place after systolic and over-systolic occlusion pressures. The slopes in nTHI at 20 mmHg were different from the slopes at occlusions ranging between 40–80 mmHg ($p < 0.001$), but they were not different from occlusions exceeding 100 mmHg ($p > 0.05$). The slopes estimated from optical densities during 20 mmHg occlusions were different from all the other occlusions ($p < 0.001$), except systolic and over-systolic occlusions. The slopes between 60 and 100 mmHg were not different between each other ($p > 0.05$). In this range however, only the slopes at 80 mmHg were significantly different from the slopes at 40 mmHg occlusion. This also suggests a maximal slope during occlusion pressures in the range 60–100 mmHg.

## 4. Discussion

The results in this study showed the simultaneous behavior of the four different techniques during vascular challenges. The TOI measured by NIRS proved to be sensitive to changes in

**Table 1.** Mean changes (±SD) and p-values for TOI, nTHI, nFlux, nPI, and $SpO_2$ during protocol 2.

| | Occlusion steps (mmHg) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Baseline | 20 | 40 | 60 | 80 | 100 | Systolic | Total |
| TOI | 70.05 ± 4.58 | 67.43 ± 5.80[c] | 67.80 ± 4.72[c] | 67.57 ± 5.04[c] | 66.73 ± 5.46[c] | 65.86 ± 4.42[c] | 67.09 ± 4.35[c] | 67.34 ± 5.43[c] |
| nTHI | 1.00 ± 0.06 | 1.05 ± 0.13[c] | 1.11 ± 0.17[c] | 1.10 ± 0.18[c] | 1.10 ± 0.19[c] | 1.11 ± 0.23[c] | 1.06 ± 0.14[c] | 1.04 ± 0.15[c] |
| nFlux | 0.95 ± 0.22 | 0.70 ± 0.3[c] | 0.49 ± 0.33[c] | 0.49 ± 0.32[c] | 0.48 ± 0.24[c] | 0.48 ± 0.21[c] | 0.24 ± 0.23[c] | 0.25 ± 0.23[c] |
| nPI | 0.81 ± 0.24 | 0.84 ± 0.50[d] | 0.78 ± 0.36[d] | 0.76 ± 0.26[c] | 0.57 ± 0.25[c] | 0.19 ± 0.16[c] | 0.03 ± 0.09[c] | 0.03 ± 0.12[c] |
| $SpO_2$[a] | 97.82 ± 1.05 | 97.81 ± 1.02[d] | 97.80 ± 1.19[d] | 97.78 ± 1.31[d] | 97.77 ± 1.45[c] | 96.88 ± 1.14[c] | — | — |
| $SpO_2$[b] | 97.29 ± 2.76 | 97.19 ± 2.86[d] | 98.42 ± 2.43[d] | 98.97 ± 2.80[d] | 98.81 ± 4.31[d] | 95.72 ± 9.61[c] | — | — |

[a] Commercial $SpO_2$.
[b] Custom-made $SpO_2$.
[c] Significant change from baseline ($p < 0.05$).
[d] Not significant change from baseline ($p > 0.05$).

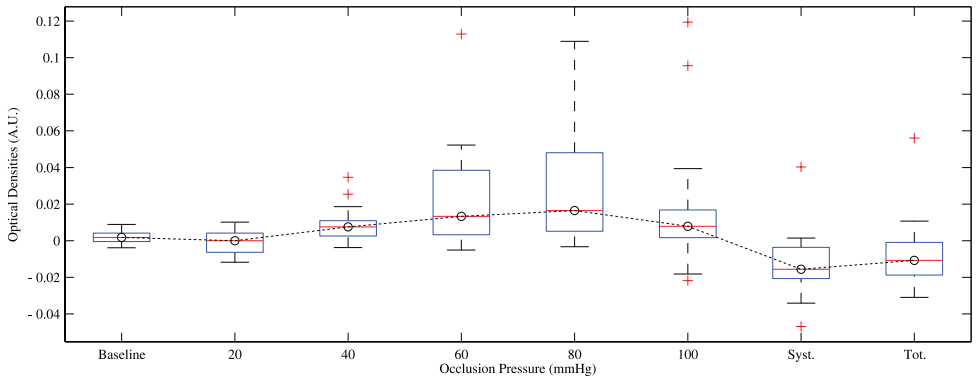


**Figure 4.** Box and Whiskers plot of the mean changes in optical densities estimated from finger PPG during protocol 2. Mean values were computed from the last 30 s of each occlusion. Syst.: systolic pressure. Total: total occlusion (over-systolic).

perfusion in both protocols. In all the volunteers, occlusion of arterial vasculature induced a more severe decrease of TOI from baseline values (i.e. steeper desaturation slope). As the TOI is directly calculated from oxygenated and reduced haemoglobin concentration changes (Pellicer and del Carmen Bravo 2011), it responded to venous blood engorgement (i.e. increase in both oxygenated and reduced haemoglobin) and over-systolic occlusion (drop in oxygenated haemoglobin and increase in reduced haemoglobin). Even though haemoglobins responses measured by NIRS differentiate between venous and arterial occlusion, we decided to analyze TOI and nTHI responses only. Similar to TOI, the nTHI indicated changes in blood volume during venous and total occlusions. Venous blood pooling caused the nTHI to increase constantly during venous occlusion until release of the cuff, while over-systolic occlusion did not produce significant changes (i.e. constant blood volume). The results from the slopes estimated from nTHI indicated a maximal response of nTHI during occlusions ranging between 40–80 mmHg. The marginal increase in blood volume at the beginning of systolic and total occlusion should be considered as a methodological limitation in the study, since manual inflations lasted for 3–4 s before achieving complete occlusion. TOI and nTHI have also further potentials as, by application of SRS, the skin contribution to the signal is minimized, and only information on pure deep tissues (muscle) is provided (Suzuki *et al* 1999).

In protocol 1, nFlux indicated changes from the baseline in both venous and total occlusion. Venous occlusion caused the mean normalized values to drop to half of baseline, with residual signal to be attributed to remaining RBC mobility (arterial inflow). The adjunction of arterial occlusion caused nFlux to reach the *biological zero*, considered as molecular Brownian motion (Choi and Bennett 2003, Binzoni *et al* 2004). The restoration of RBC flow can be observed in figure 1(c) by the hyperaemic shoot at the release of total occlusion (less pronounced after venous occlusion). The same trends in nFlux were observed in protocol 2, where the index decreased to half of baseline values in the venous occlusions between 40–100 mmHg occlusion. At systolic and over-systolic occlusion pressures, the values drastically dropped. It has been previously reported that LDF signals during venous occlusion present a slower decay, when compared to arterial occlusion, due to larger capacity of the venous circulation (Galla *et al* 1999, Yuen and Fend 2000). However, we did not observe any large delay in any of the volunteers and mean nFlux values have been computed from the last 30 s of each occlusion, once the values reached steady state. Although in our study nFlux indicated significant changes during all the steps in our protocols, differentiation between venous and arterial occlusions

may be challenging to achieve as the signal approaches the biological zero in both occlusions (Galla *et al* 1999). Moreover, LDF may be inadequate to detect venous occlusion due to its inter-subject variability, low sensitivity, and higher inaccuracies. These may be caused by intrinsic limitations of the technique (i.e. RBC concentration increasing versus RBC velocity decreasing) or limited measurement volume (Bergkvist *et al* 2015). We should also emphasize that in this study we decreased the inter-subject variability by normalizing Flux to baseline values. Thus, providing normalized values of Flux, as the nTHI in NIRS, may assist clinicians in improving detection of perfusion changes.

When near-infrared light travels into living tissues, it undergoes scattering, causing a banana-shaped light pathway. The depth of this pathway is directly proportional to the separation distance between light emitter and detector(s) (Pellicer and del Carmen Bravo 2011). Due to the large separation distance adopted in the sensors (i.e. 2.5 – 5 cm), NIRS can reach a penetration depth up to 2 cm in deep tissues (Pellicer and del Carmen Bravo 2011). If SRS is adopted, the contribution of skin and adipose tissue can be eliminated, providing information on deep tissues only (Pellicer and del Carmen Bravo 2011). Contrary to NIRS, the penetration depth of LDF is limited to only few millimeters into the skin (0.5–1.5 mm depth and a sampled area of 1 $mm^2$) (Choi and Bennett 2003, Lima and Bakker 2005, Fredriksson *et al* 2009). In this study, TOI and nTHI provided perfusion information related to deep muscle, whereas LDF assessed skin's perfusion only. Nevertheless, both modalities are considered able to provide indication of changes in perfusion and are regularly used in clinical settings for this purpose (Yuen and Fend 2000, Choi and Bennett 2003, Lima and Bakker 2005, Pellicer and del Carmen Bravo 2011, Bergkvist *et al* 2015). Even though the sampled volume is considerably different between the two techniques, the perfusion of both skin and muscle is compromised during proximal vascular occlusions. Thus, the results in this paper may provide a comparison on the ability of the techniques in detecting different degrees of vascular occlusions.

The results from $SpO_2$ measurements are not surprising and they align with previous reported results (Hallock and Rice 2003, Shafique *et al* 2012) However, our methodologies differ as we used intermittent occlusions separately and not increasing the occlusion pressure gradually in one single measurements. Even though the occlusions were short, this allowed us to investigate the different responses to different occlusions. Our results confirm that significant changes in $SpO_2$ during venous occlusion were observed in both commercial and custom-made pulse oximeters. However, these changes are still within the normoxic range (100–96%) and tolerance range of pulse oximeters ($\pm 2 - 3\%$), making difficult to distinguish venous occlusion from baseline values (Hallock and Rice 2003). Noteworthy changes in $SpO_2$ were observed only at 100 mmHg, whereas the pulse oximeters failed during both systolic and over-systolic pressure. It has to be underlined that changes in $SpO_2$ did not indicate changes in arterial blood saturation, but are indirect effects of changes in the ratio R.

The PI is commonly used in PO as a measure of adequate perfusion. The PI is represented as the ratio of pulsatile blood over the total blood volume [(ac PPG/dc PPG) $\times$ 100]. The index has been investigated as indicator of peripheral perfusion, vasodilation/constriction, sympathetic tone, or hypovolemia (Lima *et al* 2002, Lima and Bakker 2005, Sahni 2012, van Genderen *et al* 2013). To our knowledge, this is the first investigation on PI during vascular occlusions on healthy volunteers. In this study, the combined results between protocol 1 and 2 show that PI values observed in venous occlusions were statistically different from baseline. However, as can be seen in table 1, lighter venous occlusions (20–40 mmHg) did not produce significant changes. We also have to mention that the changes in PI in this study firmly reflect decreases in amplitude of the pulsatile ac component (results not presented), with little contribution of dc signals. Even though previous studies reported analogous changes in ac PPG during venous occlusions (Shafique *et al* 2012), variations in ac components - which are measure of local

arterial blood only—can also be susceptible to variations in other haemodynamics parameters (Allen 2007). Therefore, we cannot confidently conclude that ac PPG amplitudes alone (or PI), can be reliable indicators of venous congestion proximal to the finger probe.

Optical densities can be used to express changes in chromophore concentrations in a medium. In particular, optical densities that were estimated from red dc PPG component ($OD_R$) provided supplementary information on venous blood changes. We chose red PPG wavelength (660 nm) as, compared to infrared (880 nm), attenuation at this wavelength can be considered more sensitive to changes in reduced haemoglobin concentration (i.e. absorptivity of reduced haemoglobin at 660 nm is eight to ten times higher than oxygenated haemoglobin). Except at 20 mmHg, venous congestions in both protocol 1 and 2 produced increases in light attenuation as showed in figures 2 and 4. Analysis of the slopes and mean values during occlusions indicated a maximal increase of $OD_R$ in the occlusions between 60–100 mmHg, suggesting maximal venous blood congestion in this range of occlusions. During systolic and total occlusions, the $OD_R$ decreased from baseline, reflecting a decrease in blood volume. This drop is most probably due to a mechanical effect related to the absence of arterial blood flow: as the finger clip produces a slight pressure on the finger, the absence of arterial inflow causes part of blood to be pressed outside the clip. Therefore, the slopes estimated from $OD_R$ during systolic and over-systolic occlusions should be carefully interpreted as well. This limitation in the current study should be acknowledged whereas the use of pressure-free clips may help attenuate this mechanical effect. At the release of the occlusion, the hyperemic shoot and return to baseline indicated the restored arterial inflow.

## 5. Conclusion

In this study, NIRS, LDF, PPG, and PO have been investigated simultaneously during VOTs in healthy subjects. TOI, Flux, and PI were able to indicate different degrees of both venous and total occlusions. However, we cannot confirm whether Flux and PI can accurately detect venous occlusion. In addition, the optical densities estimated from red wavelength PPG traces were also able to indicate the occlusions. The optical densities from PPG may provide valuable information on venous engorgement in situations when conventional $SpO_2$ and PI may not be sufficient. Further comparative studies in healthy and disease subjects are still needed to elucidate similarities and differences between all techniques.

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