A NEW LASER DOPPLER FLOWMETER PROTOTYPE FOR MICROCIRCULATION SKIN DEPTH MONITORING

In Vitro Validation and In Vivo Preliminar Results

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Keywords: Laser Doppler flowmetry, Measurement depth, Skin microcirculation.

Abstract: A new laser Doppler flowmeter with depth discrimination capabilities is being developed to monitor skin microvascular perfusion. This new laser Doppler flowmeter is a multi-wavelength device with different spaced detection optical fibres. In order to obtain an *in vitro* validation of this prototype, measurements in two phantoms, one consisting of Teflon® microtubes and the other consisting of acrylic plates, are performed. The prototype validatation *in vivo* is also presented. Results obtained for both validations are compared with the ones obtained with a commercial laser Doppler flowmeter. The measurements show quite good agreements between both flowmeters.

1 INTRODUCTION

Laser Doppler flowmetry (LDF) is a technique for real-time and non-invasive monitoring of the microcirculation blood flow based on the Doppler Effect. In this technique, a monochromatic light beam is carried from the laser by an emitting optical fiber to the tissues under study. In the tissues, the light can be reflected, scattered, absorbed or transmitted. When photons hit moving red blood cells (RBCs) a change in wavelength occurs (Doppler shift), while photons that hit static objects have an unchanged wavelength. The magnitude and frequency distribution of these changes in wavelength are directly related to the number and velocity of the RBCs in the sampled volume. The backscattered Doppler shifted and non-Doppler shifted photons are detected and they will produce a stochastic photocurrent in the photodetector. This photocurrent contains information on velocity and concentration of RBCs (Bonner and Nossal, 1981).

LDF can be used for skin microcirculation monitoring. Skin microcirculation is present in the dermis, and it is organized into two horizontal plexuses: the most superficial is situated in the papillary dermis at 0.4 - 0.5 mm below the skin surface; the second plexus is located at the dermal subcutaneous interface at 1.9 mm from the skin surface where arteriovenous anastomoses can be found (Brevarman, 2000).

Currently, LDF human skin measurements lack in estimating the sampling depth. These difficulties lead to ambiguities in the discrimination of the fraction of light scattered from superficial and deeper blood microcirculation skin layers (Oliveira *et al.*, 2011). Besides this, commercial available flowmeters use different signal processing algorithms and calibration procedures making impossible the comparison of their results.

The most commonly used laser wavelength in LDF monitors is 780 nm and the most used fibre separation is 0.25 mm. Some studies proposed the use of other wavelengths and fibre separations in order to modify the sampling depth of the LDF technique (see for example Larsson *et al.,* 2002; Murray *et al.,* 2004). However, further work is required to have better knowledge and control over the mean sampling depth (and hence volume) in skin tissue.

Figueiras E., Campos R., Oliveira R., F. Requicha Ferreira L. and Humeau-Heurtier A..
A NEW LASER DOPPLER FLOWMETER PROTOTYPE FOR MICROCIRCULATION SKIN DEPTH MONITORING - In Vitro Validation and In Vivo Preliminar Results.
DOI: 10.5220/0003765401540159
In *Proceedings of the International Conference on Biomedical Electronics and Devices* (BIODEVICES-2012), pages 154-159
ISBN: 978-989-8425-91-1
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Besides that, only few papers reported the influence of the fibre distance and wavelength *in vivo*, and simultaneously (Gush *et al.*, 1984; Freccero *et al.*, 2006). Gush *et al.* (1984) used wavelengths in the visible spectrum where the light penetration is very influenced by the tissue optical properties. Very large fibre distances were used, and were recorded separately as the probe used has only one collecting fibre, that was placed at a certain distance from the emitting fibre before each acquisition. In Freccero *et al.* (2006) different flowmeter apparatus are compared. When different devices are compared, there are instrumental factors whose effects cannot be precisely judged (Freccero *et al.*, 2006).

We present herein a new Doppler flowmeter prototype that can bring depth discrimination information to the skin blood perfusion measurements. Validation tests are performed *in vitro* and *in vivo* and the preliminary results are compared with the ones obtained with a commercial laser Doppler flowmeter (Periflux 5000, Perimed, Sweden).

2 MATERIALS AND METHODS

2.1 Prototype

A new prototype with depth discrimination capabilities is being built in order to discriminate between different microcirculation skin layers (see figure 1).

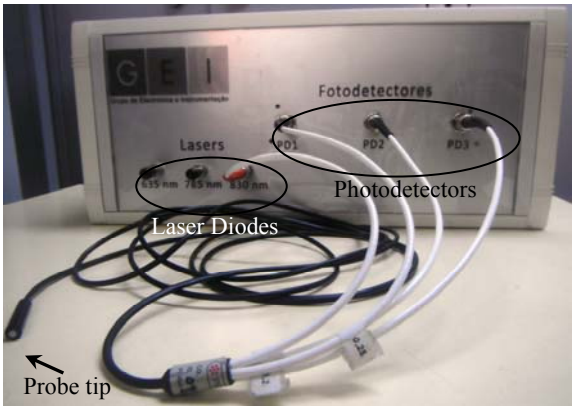

Figure 1: Laser Doppler flowmeter prototype.

The system has three constant power laser diodes drivers to supply three laser diodes of 635, 785 and 830 nm wavelength. The probe used [from Perimed (Sweden)] has a central emitting fibre and several collecting fibres located at 0.14, 0.25 and 1.2 mm from the emitting fibre. Three bi-cell photodetectors (PD 1, PD 2 and PD 3) are used for backscattered light detection (Oliveira *et al.,* 2011). The prototype has also a calibration system which provides the light intensity for the photodetectors calibration. This calibration system consists of three light emitting diodes (LEDs), with the three wavelengths used, with variable current sources (Oliveira *et al.*, 2011).

2.2 Measured Variables

The conventional parameters obtained from laser Doppler technique are blood perfusion (Perf) and concentration of moving red blood cells (CMBC).

Perf and CMBC can be estimated from the Doppler power spectrum (Bonner and Nossal, 1981). The CMBC is proportional to the zero order moment of the power spectrum P(ω) of the AC component of the light:

$$CMBC \propto \int P(w)dw \quad (1)$$

and Perf is calculated as the first order moment of the power spectrum of the AC component of the light:

$$Perf \propto \int wP(w)\, d(\omega) \quad (2)$$

In our work, the voltage signal is sampled at 50 kHz and the digitalised signal is then processed to give as an output a parameter proportional to the average Perf.

2.3 Calibration and Normalization

The calibration is performed through two steps. First, a LED illuminates the photodetector surface with a number of selected DC intensity levels. The intensity is regulated by software, where the DC level of the photodetectors is used as feedback in order to produce a stepwise, linearly increasing intensity function (Oliveira *et al.*, 2011). A block of 2048 points is acquired in each DC step and the Perf is computed for each step. The blood perfusion obtained during the DC steps is fitted to a first order polynomial, called the detector noise curve:

$$Perf_{noise} = m \times DC + b \quad (3)$$

where m is the slope of the curve, b is the y-axis value and DC is the DC voltage value. This curve is subtracted to the blood perfusion measurements.

For the second step, the perfusion in a motility standard solution (Perimed, Sweden) is scaled with a constant, M, in order to obtain 250 PU.

In order to make the perfusion independent of the total light intensity at the detector surface, it is

normalized with the factor $1/DC^2$. The normalized perfusion formula can then be expressed as:

$$Perfusion = \frac{M}{DC^2}\left(Perf - Perf_{noise}\right) \quad (4)$$

2.4 *In vitro* Validation

In vitro validation was performed in order to evaluate the performance of the prototype to different scatterer concentrations and velocities.

For *in vitro* validation two phantoms have been used. One possesses six layers of Teflon® microtubes with internal diameter of 0.30 mm and an external diameter of 0.76 mm. The other phantom consists of two glued acrylic plates with a 4×5 mm^2 excavated depression in one of them, used to study the linearity of the prototype related to different scatterer velocities and concentrations. Milk was pumped through the microtube, in the Teflon® phantom, or through the depression in the acrylic phantom. Commercial skimmed milk has been chosen as a moving fluid, because it has various components that act as scatterers (Waterworth *et al.*, 1995).

The milk was pumped with a motorized syringe at 1.56, 3.12, 4.68, 6.25, 7.78 and 9.35 mm/s in the Teflon® phantom and at 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5 and 6.0 mm/s in the acrylic phantom. Measurements were taken with milk, and with two different aqueous milk solutions (50% and 25%) during ten minutes with Perimed probe positioned perpendicular to the surface of the phantom. The mean signal for each velocity and concentration was computed for three minutes of blood perfusion signal and a linear regression study was performed.

The same protocol was executed with the commercial Periflux 5000 flowmeter, from Perimed, and with the prototype in order to compare the two flowmeters.

2.5 *In vivo* Validation

The prototype was also tested *in vivo* in healthy non-smoking subjects. The subjects were asked to refrain from drinking coffee during the measurements day. Perfusion has been recorded during thirty three minutes in the forearm with the subjects in the supine position: baseline blood flux was recorded for 20 min. Then, an arterial occlusion test was performed with a pressure cuff placed around the upper limb, inflated for 3 min at 200 mmHg. The cuff was then released to obtain a post-occlusive hyperemia and the signal was recorded during 10 min after the release of the occlusion.

For each subject, the protocol was repeated using the three laser diodes existing in the prototype: 635, 785 and 830 nm, and also for the commercial flowmeter, Periflux 5000 from Perimed, with the probe positioned in the same position during the four measurements.

The Ethics Committees of the Centro Cirúrgico de Coimbra (CCC) in Portugal, approved this study. Informed consent was obtained from the subjects before the recordings were made. The measurements were made in 20 subjects and the preliminary results are presented.

3 RESULTS AND DISCUSSION

3.1 Calibration

The steps obtained with our prototype during the calibration with the 830 nm LED are shown in figure 2 where a pair of signals was obtained for each photodetector (as the detection system is made by bi-cell photodetetors). It can be seen that the intensity level pairs diverge when the voltage increases. This is due to the lack of alignment between the photodetectors and the LEDs.

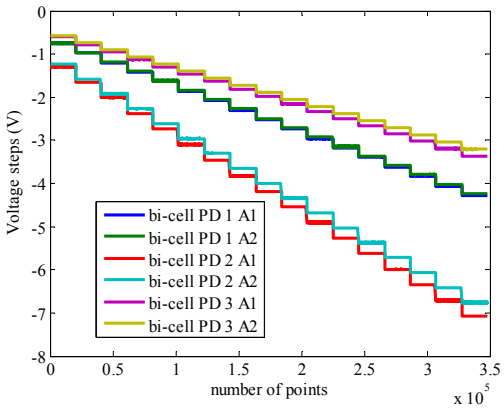

Figure 2: Photocurrent generated by the three bi-cell photodetectors during calibration: PD 1 A1 and A2 are the PD 1 signals, PD 2 A1 and A2 are the PD 2 signals, PD 3 A1 and A2 are the PD 3 signals.

The detector noise perfusion curve is presented in figure 3. The slope of the curves are (-6.86, -6.67 and -6.73)x10^{-5} for PD 1, PD 2 and PD 3, respectively and the y-axes value are (0.157, 0.165 and 0.162)x10^{-3} with a coefficent of determination, R^2, close to one.

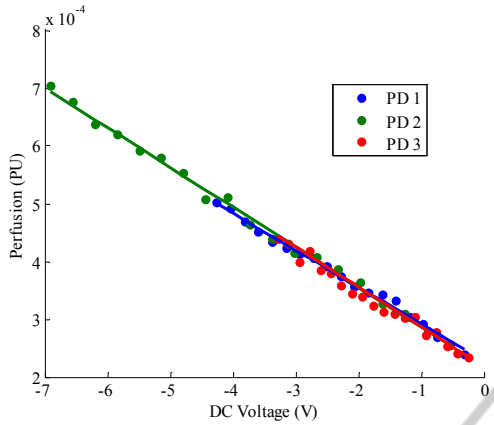

Figure 3: Detector noise perfusion curves obtained for each photodetector: PD 1, PD 2 and PD 3.

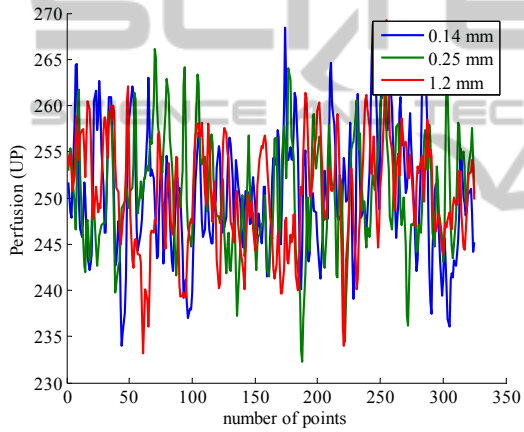

Figure 4: Perfusion obtained in the motility standard with the prototype for a 785 nm laser light for the three fibre distances.

The perfusion obtained in the motility standard solution after the calibration for the 785 nm laser light is presented in figure 4 with a mean value of 250.6± 6.8, 250.5± 6.1 and 250.4± 6.0 for 0.14, 0.25 and 1.2 mm fibre distances, respectively. These values are in the range of the ones obtained with the Perimed flowmeter which can be 250±15.

3.2 *In vitro* Validation

3.2.1 Periflux 5000 - Teflon® Phantom

In the Teflon® phantom, results obtained with Periflux 5000 show that the perfusion increases with the velocity and concentration of milk and with the emitting-receiving fibre distance. Non-linearities were found for 0.14 and 0.25 mm fibre separations. The results obtained for all velocities and the three emitting-receiving fibre distances at 25% milk concentration are presented in figure 5. Perfusion saturates for 6.25 mm/s in milk using the 1.2 mm fibre separation. A good fitness between the linear model and the obtained results was obtained in the linear regression analysis: R^2 was always close to one and the p-values were always smaller than 0.01, except for results collected with 1.2 mm fibre distance in milk. In this case it was only considered three samples for the regression analysis (due to the saturation mentioned above).

The saturation of the perfusion can be explained by the fact that the Periflux 5000 flowmeter is for perfusion measurements in living tissues and the phantom does not perfectly mimic them.

Positioning the probe in the top of the microtube Teflon®-based phantom was difficult due to the microtube curvature. This, together with the small milk volume in the microtube, when compared with the tube volume, lead to the sub-estimation and uncertainties of the perfusion measurements. These factors could be the reason for the non-linearity obtained. That is why an acrylic phantom was developed 1) with a plain surface easing the contact probe/phantom and 2) with a higher volume of milk.

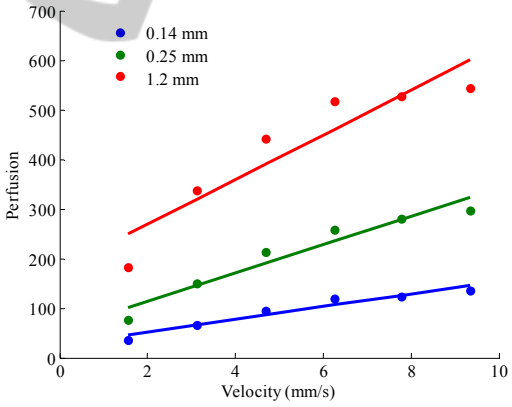

Figure 5: Perfusion obtained in the Teflon® phantom with the Periflux 5000 flowmeter (Perimed, Sweden) for all velocities at 25% aqueous milk solution.

3.2.2 Prototype - Teflon® Phantom

Some drawbacks in the prototype results obtained in the Teflon phantom were found, resulting from the positioning difficulties of the probe in the top of the microtube. These drawbacks are difficult to overcome as real time signal processing is not yet implemented in the prototype.

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3.2.3 Periflux 5000 - Acrylic Phantom

For the acrylic phantom, perfusion increases with the velocity and with the concentration of the moving fluid for each emitting-receiving fibre distance. Moreover, we also noted that increasing the emitting-receiving fibre distance leads to a larger perfusion value. This is due to the larger volume measured with larger emitting-receiving fibre separations. For the 1.2 mm emitting-collecting fibre distance, perfusion saturates for the higher velocities. The statistical analysis showed good correlation between the fitted model and the results since R^2 was close to one and the p-value was lower than 0.01. Therefore, we can conclude that the relation between perfusion and velocity is linear, as expected.

The perfusions obtained with the Periflux 5000 flowmeter in the acrylic phantom with 1.2 mm emitting-fibre distance are shown in figure 6. It can be seen that the perfusion saturates at 3.5, 4 and 4.5 mm/s for milk, 50 and 25% aqueous milk solution, respectively.

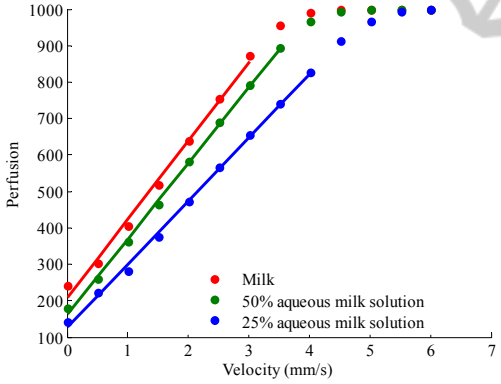

Figure 6: Perfusion obtained in the acrylic phantom with the Periflux 5000 flowmeter (Perimed, Sweden) for all velocities with the 1.2 mm emitting-receiving fibre distance.

3.2.4 Prototype - Acrylic Phantom

In the acrylic phantom, results obtained with the prototype show an increase of the perfusion with milk velocity for all wavelengths of incoming light, as it was expected. Moreover, perfusion also increases with the emitting-receiving fibre distance. Concerning the milk concentration, for the three laser light wavelengths, the perfusion increases with milk concentrations for each fibre distance and velocity. To investigate if the relationship between perfusion and milk velocity was linear, a linear fitting was performed. R^2 values obtained were higher than 0.94 and the p-values were always lower than 0.01. Therefore, we can conclude that our perfusion measurements are linearly proportional to the velocity of the moving fluid.

Perfusion obtained in the acrylic phantom with 785 nm laser light for all velocities with the 1.2 mm emitting-receiving fibre distance are shown in figure 7.

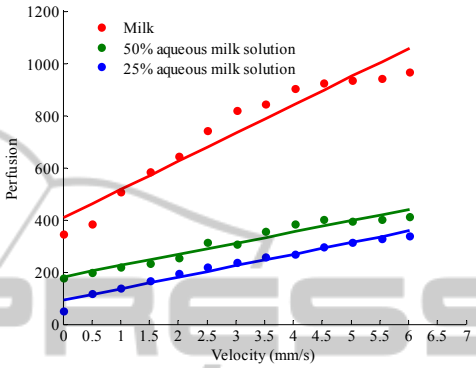

Figure 7: Perfusion obtained in the acrylic phantom with the prototype for a 785 nm laser light for all velocities and with the 1.2 mm emitting-receiving fibre distance.

3.3 *In vivo* Validation – Preliminary Results

The preliminary *in vivo* results are in accordance with the literature and with the commercial prototype results. The results obtained for one subject using the 635 nm laser light of the prototype and the commercial flowmeter are shown in figures 8 and 9. The baseline blood flux was recorded for 20 min (T1). During the occlusion, perfusion decreases for any fibre distance (T2). After the occlusion release the reactive hyperaemia peak, P, occurs and then the signal returns to the baseline value (T3).

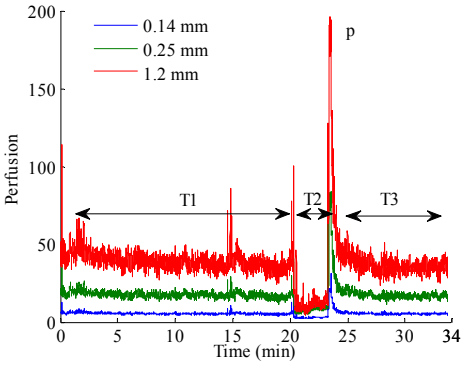

Figure 8: Signal collected in the human forearm with the non-invasive prototype with a 635 nm laser diode. The results for the three fiber distances are shown.

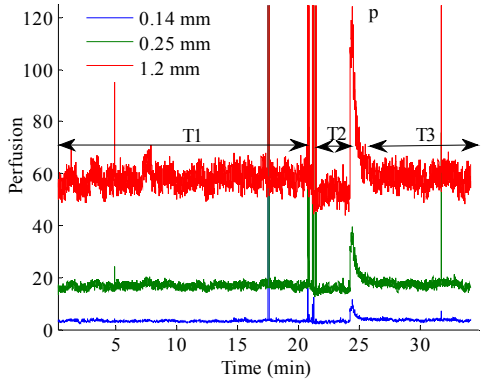

Figure 9: Signal collected in the human forearm with the Periflux 5000 flowmeter (Perimed, Sweden). The results for the three fiber distances are shown.

4 CONCLUSIONS

Calibration was performed with success despite the lack of alignment between the LEDs and the PDs. In general, there are good agreement between the *in vitro* results of the Periflux 5000 flowmeter and our prototype taken on the acrylic phantom. In both systems Perf increases with the increasing of the parameters under evaluation, i.e, velocity, milk concentration and emitting-receiving fibre distance. There is only a mismatch with the theoretical expectations for the 635 nm laser diode when blood perfusion of different milk solutions are compared for the signals collected in the Teflon® phantom. Furthermore, it was statistically proved that Perf is linearly proportional to the velocity of the moving fluid, as theoretical principles indicate. Moreover, *in vivo* preliminary results obtained with the non-invasive prototype are in accordance with the literature and with the commercial flowmeter used.

ACKNOWLEDGEMENTS

The authors thank the “Instituto de Investigação Interdisciplinar (III)” of the University of Coimbra, “Acções Universitárias Integradas Luso–Francesas” (PAUILF) programme and “Fundação para a Ciência e a Tecnologia (FCT), Lisbon”, for supporting this work.

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