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In Vivo, Depth Resolved Measurement of Blood Flow with Time Domain Diffuse Correlation Spectroscopy

M. Pagliazzi[1], S. Konugolu Venkata Sekar[2], L. Colombo[1,2], E. Martinenghi[2], J. Minnema[1], R. Erdmann[3], D. Contini[2], A. Dalla Mora[2], A. Torricelli[2], A. Pifferi[2,4], & T. Durduran[1,5]

1)ICFO-Institut de Ciències Fotòniques, The Barcelona Institute of Science and Technology, 08860 Castelldefels (Barcelona), Spain; 2)Politecnico di Milano, Dipartimento di Fisica, 20133 Milano, Italy; 3)PicoQuant GmbH, 12489 Berlin, Germany; 4)Istituto di Fotonica e Nanotecnologie, Consiglio Nazionale delle Ricerche, 20133 Milano, Italy; 5)Institució Catalana de Recerca i Estudis Avançats (ICREA), 08015 Barcelona, Spain

Abstract: We have achieved continuous path length resolved diffuse correlation spectroscopy *in vivo* by means of an actively mode-locked Ti:Sapphire laser that allows high coherence pulses, thus enabling adequate signal-to-noise ratio in relatively fast (~1s) temporal resolution.

OCIS codes: (170.5280) Photon migration; (170.6920) Time-resolved imaging; (170.3890) Medical optics instrumentation.

1. Introduction

Diffuse correlation spectroscopy (DCS), combined with time-resolved reflectance spectroscopy (TRS) or frequency domain spectroscopy, aims at path length (i.e. depth) resolved, non-invasive and simultaneous assessment of tissue composition and blood flow. However, while TRS provides a path length resolved data, the standard DCS does not.

Recently, a time domain DCS experiment [1] showed path length resolved measurements with narrow time gates for improved quantification with respect to classical DCS, but was limited to phantoms and small animal studies. We have demonstrated, with an appropriately large coherence length pulsed laser, that by studying the auto-correlation of the photons within a broader time of flight (TOF) gate, we can improve the signal-to-noise ratio, allowing for in vivo measurements on adults with high (1 s) time resolution [2].

We envisage three main advantages of the time-domain DCS approach as compared to standard continuous wave (CW) DCS. First, the time domain approach will add one further variable – time – which can be exploited to select photons with increasing depth sensitivities [3]. Second, time-domain DCS also offers an elegant physical model that separates the distribution of time of flight of the photons in tissue and the phase shifts due to multiple scattering from those due to moving scatterers, for better quantitation of blood flow. Third, this approach allows for a simultaneous measurement of the time-domain NIRS data types and DCS, thus providing a complete picture of blood hemodynamics.

Here, we present our advances in phantom and for *in vivo* studies on the adult, on the forehead and the arm.

2. Materials and methods

We have used a custom made, high temporal coherence pulsed Ti:Sapphire laser operated in active mode-locked regime at a wavelength λ=785 nm. The pulses repetition rate was 100 MHz. We achieved synchronization with a time correlated single photon counter (TCSPC) module (PicoHarp 300, PicoQuant, Berlin, Germany) by splitting off a small fraction (<5%) of its light to a photodiode (PDM400 Becker & Hickl, Germany). We used shaping electronics to adapt the detected signal to the requirements of the TCSPC (100 MHz to 33 MHz). A 4 μm core single mode fiber was used for collection that channeled the light to a single-photon avalanche diode (SPAD) detector (PDM, Micro Photon Devices, Italy).

Fig. 1 shows the experimental setup. The detailed description of the experimental setup is given elsewhere [3].

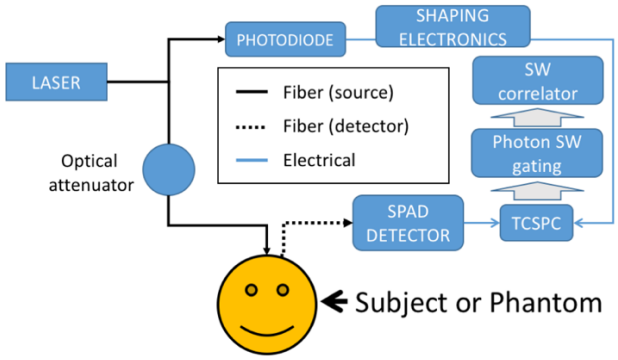

Fig. 1 Schematics of the experimental setup. See text for details.

The FoCuS-point software correlator [4] was used for the post-processing of the data. We have adapted it to correlate the time stamps of the arrival time of the detected photons, that were recorded by the TCSPC, within a time window (gate) $[t_A, t_B]$ with respect to the laser emission time (t_0). Earlier gates allow us to achieve more sensitivity to the shallow layers of the sample, while layers that lay deeper with respect to the source-detector plane have a greater influence on the later gates.

One of the biggest challenges is to quantify the motility of the scatterers in the different gates. The Siegert relation $g_2(\tau) = 1 + \beta|g_1(\tau)|^2$ relates the measured normalized intensity auto-correlation function $g_2(\tau)$ to the normalized electric field auto-correlation function, $g_1(\tau)$. Here, τ is the correlation delay time and β is a constant, determined by the collection optics. Once we estimated $g_1(\tau)$, we fit to the numeric evaluation of the integral formula first developed by Yodh et al. [5]

$$g_1(k,\tau) = \int_{t_A}^{t_B} P(t)\, e^{-k' BFI\, \tau t}\, dt$$

to obtain the blood flow index (BFI). Here, $P(t)$ is the expected distribution of times-of-flight (TOFs) of photons in tissue and is known along with the optical properties of the sample [6]. The integration is between the extremes of the chosen temporal gate. The constant k' is proportional to the scattering coefficient, the speed of light in the medium and inversely proportional to the square of λ.

We have used two broad gates and we have chosen their extremes based on the shape of the distribution of TOFs (DTOF) that we measured case by case. The early gate extended from t_0 to the time at which the measured DTOF dropped to 90% on the falling edge of the curve, with respect to its maximum, for a total width of about 800 ps. The late gate extends from this point to the time at which the DTOF is no longer distinguishable from the noise floor, which results in a width of between 2 and 3 ns.

For all the *in vivo* studies, the laser power was limited by using an optical attenuator to meet the maximum permissible exposure limit for human skin. The protocol was approved by the Ethical Committee of Politecnico di Milano and it was conducted in agreement with the Declaration of Helsinki. All subjects gave written consent before their participation.

3. Results

Measurements on a layered phantoms show a more marked decrease of BFI in the early gate with respect to the late gate when the superficial layer is filled with a liquid of the same optical properties but with a lower particle diffusion coefficient. In particular, BFI is 53% smaller in the early compared to the late gate for a 5 mm thickness with reduced particle diffusion coefficient.

On the head of a healthy adult subject we have placed source and detector fibers spaced by 1 cm on the frontal region. By applying moderate pressure to hinder the flow of blood in the superficial

scalp and skull regions we see a more marked decrease of the BFI in an early with respect to a late gate (see Fig. 2, a). We have acquired for 300 s and recorded a total of $2.7 \cdot 10^7$ photons.

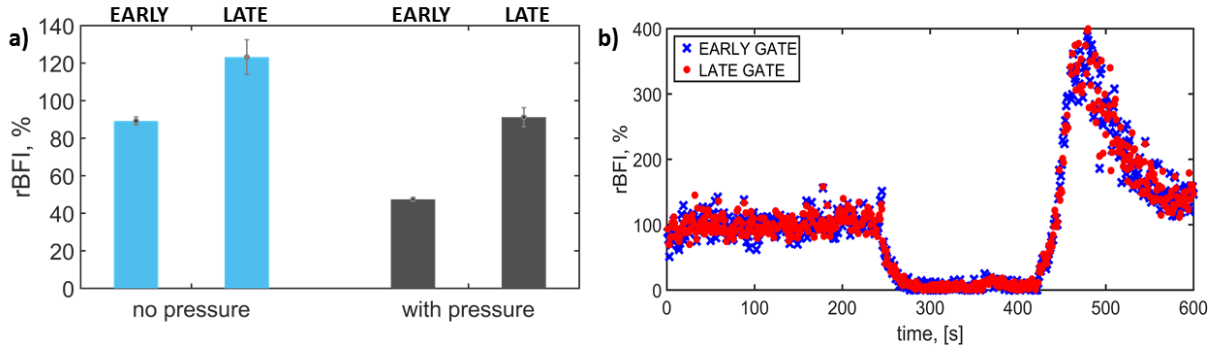

Fig. 2 a) BFI measured in early and late gates normalized to the [0,∞] gate (in other words, all the photons were used for correlation); on the left, no pressure was applied, while on the right, a moderate mechanical pressure was applied on the probe to hinder the superficial blood flow. b) Percentage BFI (relative to first 100 s baseline) variations during an arterial cuff occlusion in the arm, measured in the two gates separately.

The high value of β (0.36 and 0.32 for the early and late gates, respectively) and the photon detection rate that we have achieved during an arterial cuff occlusion of the arm of a healthy adult subject allowed us to measure the shallow and deep variations of BFI with 1 s time resolution (see Fig. 2, b). Source and detector fibers were placed on the brachioradialis muscle at 1 cm separation and $3.3 \cdot 10^7$ photons were recorded in 600 seconds.

4. Discussion and conclusions

In a two-layer phantom, we have observed a selective drop in the typical decorrelation time corresponding to the photons arriving early to the detector when they cross a superficial region with reduce particle Brownian diffusion coefficient.

The reduction in the BFI as we apply mechanical pressure from outside to compress the superficial tissue of the head, shows that we do take advantage of the multiple gate strategy to achieve depth resolution with a single-shot measurement at a relatively short source detector separation (1 cm). Our results reproduce previous assessments of the extra cerebral layer contribution to the cerebral BFI in DCS [7,8]. Instead of using multiple source-detector separations, we have have achieved depth resolution by the time gating only.

We were able to obtain 1 s resolution when monitoring fast BFI changes of 500% in the arm during arterial cuff occlusion and reperfusion. However, we believe that too thin superficial layer (2.5mm, measured by skin caliper) of the subject prevented us to detect higher hyperemic peak in the deeper muscle as expected.

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